SHOCK AND CIRCULATORY HOMEOSTASIS

Transactions of the Second Conference
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THE JOSIAH MACY, JR. FOUNDATION CONFERENCE PROGRAM

As an introduction to these Transactions of the Second Conference on Shock and Circulatory Homeostasis, I should like to outline what it is that the Foundation hopes to accomplish by its Conference Program. We are interested first of all, in furthering knowledge in a particular field, i.e., shock and circulatory homeostasis, and to this end the participants were brought together to exchange ideas, experiences, data, and methods. In addition to this particular goal, however, there is a further, and perhaps more fundamental, aim which is shared by all our conference groups. This is the promotion of meaningful communication between scientific disciplines.

The problem of communication between disciplines we feel to be a very real and urgent one, the most effective advancement of the whole of science being to a large extent dependent upon it. Because of the accelerating rate at which new knowledge is accumulating, and because discoveries in one field so often result from information gained in quite another, channels must be established for the most effective dissemination and exchange of this knowledge.

The increasing realization that nature itself recognizes no boundaries makes it evident that the continued isolation of the several branches of science is a serious obstacle to scientific progress. Particularly is it true in medicine that the limited view through the lens of one discipline is no longer enough. For example, today medicine must be well versed in nuclear physics because of the tracer techniques and the injury which can result from radiation. At the other extreme, medicine is certainly a social science and, through mental health, must be concerned with economic and social questions. The answer, then, is not further fragmentation into increasingly isolated specialties, disciplines, and departments, but the integration of science and scientific knowledge for the enrichment of all branches. This integration, we feel, can be encouraged by providing opportunities for a multiprofessional approach to given topics.

Although the fertility of the multidiscipline approach is recognized, adequate provision is not made for it by our universities, scientific societies, and journals. And perhaps the presence of other

hindering factors must be admitted Partly semantic in nature, they may also to some degree be psychological. Admittedly, it is oftentimes difficult to accept data derived from methods with which one is unfamiliar. By making free and informal discussion the central core of our meetings, we hope to achieve an atmosphere which minimizes as much as possible these semantic and emotional barriers

Thus, our conferences are in contrast to the usual scientific gatherings Presentations are designed not to present neat solutions to tidy problems, but rather to elicit provocative discussion of the difficulties which are being encountered in research and practice We ask that the presentations be relatively brief, and emphasis is placed upon discussion as the heart of the meeting Our hope is that the participants will come prepared not to defend a single point of view but, with open minds, to take full advantage of the meeting as an opportunity to speak with representatives of other disciplines in much the same way as they talk with their own colleagues in their own laboratories

During 1953 under the Conference Program conferences will be held on the following topics. administrative medicine, adrenal cortex, aging, connective tissues, consciousness, cybernetics, infancy and childhood, liver injury, metabolic interrelations, nerve impulse, renal function, and shock and circulatory homeostasis.

When a new conference group is organized, the Chairman, in consultation with the Foundation, selects fifteen scientists to be the nucleus of the group which will hold annual meetings for a period of five years Every effort is made to include representatives from all pertinent disciplines From time to time, however, new members are added by the group to fill gaps in viewpoint or techniques A small number of guests is invited to attend each meeting, but, for the purposes of promoting full participation by all members and guests, attendance at any meeting is limited to twenty-five During a conference's prescribed lifetime we cannot possibly include more than a small fraction of the key investigators in the field, and one of the difficulties in forming a group, such as this one on shock and circulatory homeostasis, is that it is necessary to exclude so many investigators we should like to include

The transactions of these meetings are recorded and published. This is done because the Foundation wishes to make current thinking in a field available to all those working in it, and to those in other fields who are concerned with science, for example, government officials, administrators, etc. Logic is a vital aspect of science,

but equally essential is the intuitive or creative aspect Research is as creative as the painting of a portrait or the composing of a symphony Although logic is, of course, necessary in order to rearrange, to test, and to validate, research thrives on creativity which has its source in unconscious, nonrational processes Unfortunately, however, in the research reports which are presented to the world in scientific journals, this integral part of scientific endeavor is shriveled by the cold, white light of logic By preserving the informality of our conferences in the published transactions, we hope to portray more accurately what goes on in the minds of scientists and to give a truer picture of the role which creativity plays in scientific research

FRANK FREMONT-SMITH, M D,
Medical Director

INTRODUCTORY REMARKS

EPHRAIM SHORR
Chairman

I AM GOING to ask each person, not only for the sake of the readers but for the sake of his friends here, to identify himself and to tell us just why he is interested in the field of circulatory homeostasis. In the past, we have been extraordinarily cryptic in our biographies, and perhaps our readers would have liked to know a little more If I may ask you to overcome your natural shyness and modesty, and regard it as a service to assist the public in learning what is responsible for the evolution of a vascular physiologist, I think the Josiah Macy, Jr Foundation will be pleased, and also we shall all know each other a little better

MELVIN H. KNISELY: A number of individual men and specific experiences have had a great deal to do with shaping my habit patterns and character My father, Samuel H Knisely, was the first person and one of the strongest influences When I was a small boy, my father took me on many trips by rivers or lakes or through forests We usually carried a rifle Constantly, he said to me, "What do you see, Son?" And after I had told him what I saw, he said, "What do you think about it?" That established a life pattern of always observing and always wondering what the observations meant Not until I was studying pathology under Prof Paul Cannon at the University of Chicago did I again come under the hand of a man who insisted upon the separation of observations from deductions Obviously, a sharp, hard separation of observation from deduction is a major part of the scientific game And, owing to this early period with my father, I grew up with that habit pattern established

A high school teacher, a Mr Tenhaven, taught me physics, making it utterly delightful, intriguing, and fascinating He taught physics by having us work with equipment, simultaneously learning the principles incorporated into the design of the equipment, and thus, without know-

ing it, I became consumed with the idea that a machine has to be constructed in such a fashion that its parts can work Each and every machine must be so constructed that working in cooperation, the functions of the machine will be carried out

For three years, I myself taught elementary physics in a high school, and at the end of that time I was thoroughly grounded in almost all Newtonian physics Consequently, when I entered medical school at the University of Chicago in the fall of 1930, an astonishing thing happened The anatomical courses were loaded with Latin and Greek terminology The ideas presented were largely embroidered with deductions concerning how the parts of the body might work, or how it was seriously believed that they would work Much to my astonishment, the imaginings had largely been done by men who obviously did not know even elementary Newtonian physics This meant that the harder one studied, the farther one's mind travelled away from understanding real functions as they must be carried out in order to have the machine work If one believed the physics, so carefully constructed for four hundred years, then one could not believe great masses of maunderings which cluttered lectures and text books.

Shortly, then, under Professor Robert R Bensley, I began studying living internal organs, transilluminated so that microscopes could be put over the organs and their architecture, and behavior, and responses seen while they were alive The observations made while studying living structures are quite different than those made when the tissues are dead For instance, every living structure has living dimensions Capillaries have length, they have diameter, the flow through them occurs at finite rates. None of these dimensions ever shows up in dead tissues Thus, because the physical dimensions of parts are even today almost completely unrecorded, it is quite impossible to bring to bear on problems of microscopic physiology the whole great world of mathematics and physics, a world which functions only in terms of measurements and manipulations of measurement Curiously enough, in all histology there are almost no recorded dimensions, that is, no dimensions measured and recorded in terms of what a physicist would call dimension Almost the only dimension in histology is the diameter of the red blood cell

The frustration of conventional histology and the urge to learn how the microscopic machinery of the body operates led me into the hands of a number of the world's great teachers It is a privilege to give tribute to them. Specifically, I would mention R R. Bensley, George W. Bartelmez, Paul Cannon, Normand Hoerr, Paul Brandt-Rehberg at the University of Copenhagen, August Krogh, Victor Johnson, Ralph Gerard, Anton Carlson. Each of these men contributed knowledge, attitudes, habits of thought, and, above all, insistence upon separation of observations from deductions and upon the maintenance of a rigorously critical attitude toward one's own work.

HANS H. USSING: In recent years, I have been mostly interested in the study of the permeability of living membranes, using isotopic tracers. Since the capillary wall is one of the important membranes of the organism, and since its function is intimately related to the phenomena of shock, I am very pleased indeed to have the opportunity of attending this meeting.

OTTO LOEWI: As a youngster, I was not at all interested in medicine or the medical sciences, but only in art My parents, however, did not consider this study of art to be promising for making a living, so I switched to medicine at the University of Strasbourg I was fortunate enough to find there as my teachers persons like Naunyn, Minkowski, and Schmiedeberg

I was never aware how it happened, but during my fourth year in medical school, I decided suddenly to do some research work in the laboratory of Schmiedeberg He gave me as a topic for my thesis the study of the influence of hydrocyanic acid, phosphorus, and arsenic on the frog's heart After my graduation in 1896, I worked for a year in organic and inorganic chemistry, and for six months in biochemistry with the famous Hofmeister Then I went to Frankfurt on the Main to be an assistant to von Noorden in the city hospital for a year and a half Meanwhile, my interest in physiology had arisen, and I turned to pharmacology as drugs were known to be fine tools for the approach to physiologic problems I had the good luck to be an assistant to the great Hans Horst Meyer for a period of ten years

During my long career, I have never published a paper on the effect of a drug unless it would add to our knowledge of physiologic functions. I have always considered myself a physiologic pharmacologist or a pharmacologic physiologist, whatever term is preferred.

BENJAMIN W. ZWEIFACH: I suppose I have always had a distorted view of the circulatory apparatus, peering at it from the bottom of the ladder through the eyes of the discipline of cell life, protoplasmic structure, organization of cell surfaces, and permeability of membranes. While working under the stimulating influence of Dr. Robert Chambers, he suggested as a thesis problem the application of micromanipulative methods for a study of the Rouget cell. Having once been introduced to a new and completely fascinating world through microscopic visualization of the circulation, I have since remained intrigued by the delicate interplay of mechanisms which set off the capillary bed as a discrete functional entity. We became interested in the relation between the nature of extracellular coats and permeability of organized membranes, such as the capillary wall, and suggested a theory of capillary permeability which has stimulated considerable investigation along those lines. With the onset of World War II, we were diverted into studies of the circulatory changes during shock, with the purpose of shedding light on the so-called "toxic factor," much publicized during World War I by Cannon and Bayliss. The rat meso-appendix technique was a direct outcome of our efforts along these lines. Following World War II, several forays were made into the peripheral circulatory changes in other disease entities, such as hypertension. My present interests are along the lines of filling the lacunae in our knowledge concerning the basic aspects of capillary physiology, especially the complex mechanisms concerned with the local regulation of peripheral blood flow.

MARK NICKERSON: I am here because of an extremely dry summer in 1935. I had been working in the logging camps and mills of western Oregon, with no real expectation of going to college, but after being out of work for almost the entire summer because of the fire hazard, I decided to go back to school. I enrolled at Linfield College and had the good fortune to come under the influence of a relatively

unknown biologist by the name of Macnab, who, I believe, is one of today's outstanding teachers of biological science. In spite of a very small department and poor facilities, his personal enthusiasm for the subject led a majority of his students to undertake graduate study in the field

My first graduate work was in insect physiology and biophysics, and from this I went into experimental embryology. Finally, I entered the field of pharmacology, partly as a result of experience in the Chemical Warfare Service but particularly because of contact with two of the most stimulating men in the field, Goodman and Gilman

My current interests are in the autonomic nervous system and in the effects of drugs upon this system, particularly with regard to their specificity of action In the field of autonomic pharmacology, as in many others, a great many experiments are misinterpreted because attention is focused, purposely or fortuitously, upon a single action of a drug, with no recognition of the fact that none of the available pharmacologic tools is really one hundred per cent specific in its action Consequently, I have felt that it is necessary to obtain more information regarding the pharmacology of the autonomic drugs before the results of experiments employing these agents can be adequately interpreted in terms of the physiology of the autonomic nervous system

HAROLD D. GREEN: I suppose that I first became interested in physiology one summer when I had an opportunity to work in Dr Carl Wiggers' department at Western Reserve University After finishing my medical school and hospital work, I was invited to go back with Wiggers for a period of time, and I liked it so well that I stayed with him until 1945, when I went to my present position

One thing that Wiggers was very much interested in, of course, was the study of circulation, using pressure measurements It did seem to me a bit difficult to make many interpretations of a system in which fluid was circulating, based solely on pressure measurements, and I felt that one should try to combine flow measurements with it, if possible In 1939, I was able to spend a year at the Massachusetts Institute of Technology A good bit of time while there was devoted to the Departments of Hydraulics and Electrical Engineering, where I picked up

some ideas on flowmeters, which Dr. Gregg and I were subsequently able to put into use When I returned to Cleveland, we used those techniques in studying blood flow in shock

At Bowman Gray, we have been studying particularly the hormone factors that regulate the blood flow in the various vascular beds

FRANK L. ENGEL My main areas of interest are in the endocrine control of metabolism and the metabolic response to injury. My interest in the shock field developed quite accidentally through the process Walter Cannon has called serendipity. When I was a National Research Council Fellow in Dr C N H Long's Department of Physiological Chemistry at Yale University in 1941, I was working rather enthusiastically on a problem concerning the role of the adrenal cortex in the regulation of the metabolism of amino acids, carbohydrates, and certain carbohydrate intermediaries, particularly lactic acid and pyruvic acid In the course of carrying out these experiments on adrenalectomized animals, I noted some interesting and striking changes in the levels of amino nitrogen, lactate, and pyruvate in their blood, which at first I attempted to relate to endocrine influences After doing this for some months, I began to wonder whether the frequent sampling for the multiple blood chemical analyses might not be influencing my results Hence, I decided to do some proper controls, taking an equivalent amount of blood from normal animals to make sure this was not responsible for the results I was getting In so doing, I discovered that what I thought was the effect of the adrenal cortex on these particular metabolites was actually the effect of hemorrhagic shock Having a very wise and understanding chief, I was encouraged to go ahead and look at these changes rather than to try to figure out how to do the experiment in the proper manner to get the answer that I was initially looking for From those original findings, there began the group of studies on the metabolic changes in shock which were expanded considerably in the Department of Physiological Chemistry at Yale in the following years, stimulated by the war The first report of this work, incidentally, was at an early Macy Conference on Shock in 1943 *

*Conference on Intermediary Metabolism in Shock, held in New Haven, Conn, Nov 2, 1943 (*Privately distributed*)

Since leaving Yale, I have done very little in the field of shock except for a renewal of interest in certain aspects of it about a year ago, which I shall discuss later. I have been more interested in the early metabolic response to injury

GORDON K. MOE. I started on the cardiovascular system as a first-year graduate student, under Maurice Visscher, and never got away from it Without saying anything more about my own career, I should like to remark that Dr. Engel's statement about his inadvertent entrance into the shock problem at Yale reminds me of a cartoon which is posted on our bulletin board It pictures a dejected scientist, surrounded by retorts, condensers, and so on The caption says, "Poor boy! After twenty years of work, he finally found the answer, but now he has forgotten the question"

EUGENE A. STEAD, Jr. I suspect I am here because in 1936 Dr Soma Weiss spent twenty-four hours in the General Hospital in Cincinnati, where he looked at a lot of ill people. He was interested in how the circulation worked in those people and how it related to their symptomatology and their eventual recovery or death Under his guidance, in subsequent years, I became interested in circulatory problems, an interest that has continued until the present

DICKINSON W. RICHARDS, Jr. The group I work with arrived at the study of shock more or less by a side-door approach Having picked up the European technique of cardiac catheterization and developed it to some extent, we were using it for other purposes when the war came along. It had some application to the problems of shock, so we went to work on that The technique has added certain measurements to the study. I am not sure it has solved any fundamental problems

STANLEY E. BRADLEY: My interest in the problem of shock was inspired by work with Dr Richards and the Shock Team at Bellevue Hospital

EWALD E. SELKURT: My first interest was generated by association with two grand men in the game, Dr Meek and Dr. Eyster, at the University of Wisconsin where I was a graduate student. And I might add hastily that I went into physiology to make a living I entered Dr. Homer Smith's laboratory at New York University, where my interest in kidney physiology was generated and under whose teach-

ing and guidance I learned enough about the kidney to begin investigation and research in this field. I went to Western Reserve in 1944, during the war, and came under the great stimulus of Dr. C. J. Wiggers, who has been mentioned already by Dr. Green. He encouraged me to investigate the problem of kidney function in shock, which brought me into the field which this group is presently interested in. With that, I made certain other investigations in the field of shock, and since the war I have continued on the problem of autonomy of circulation in the kidney, the physical factors and neurogenic factors involved, and, most recently, the influence of hypoxia on certain aspects of kidney function.

GEORGE E. BURCH I have spent all my postgraduate life in studying the circulation, primarily the circulation of man. In recent years, the problem of sweat and sweating from the fingertips of man has led us into the use of isotopes to trace electrolytes in normal and abnormal states of fluid balance. At the moment, we are working primarily with radioactive isotopes. I have been wondering why I was invited here since I have never worked on shock, probably because of the lack of a good idea.

RUSSELL M. NELSON. My training and nomenclature labels me a surgeon. Under the influence of Dr. Wangensteen and Dr. Dennis, I became interested in the physiologic aspects of what happens to patients undergoing surgery. In attempting to develop an artificial heart and lung in the laboratory, we saw shock, and that is where the story began.

I think the Macy Conference is a good place to air problems encountered in shock, because the academic disciplines of medicine, physiology, biochemistry, bacteriology, and immunology are man-made. The body makes no such distinction, and I find myself involved in these various disciplines in the studies that we are doing.

JOHN W. REMINGTON As a budding endocrinologist, I came to Princeton some years ago. In our study of the adrenal cortex, we were impressed with the strong resemblance between the circulatory events seen in adrenal insufficiency and those reported for shock as seen in normal animals. That, with the impetus of the war years, led to a study of several different shock states, particularly directed toward the possible therapeutic use of cortical hormones.

Then I went to Georgia, where Dr Hamilton taught me how naive my concepts of circulatory function really were. The philosophy of his department is that even in this most worked-on field of physiology, hemodynamics, many of the fundamental precepts that we teach the students and use in guiding our own thinking are based on insecure evidence We have worked on shock not as an end in itself but in the hopes that such a study would shed light on the basic mechanisms by which the circulatory apparatus works.

G. C. COTZIAS: I was born in Greece, where people look upon research as something that only the Gods on Olympus could undertake In other words, they have a very profound respect for it and regard it as an activity requiring special mental and physical equipment

I am in a sense a war profiteer, because when the war came I was chased out of Greece and found myself on the way to the United States Luckily, since no other medical school would have me, I went to Harvard and there met Dr Joseph Aub He took me under his wing. He kept telling me that research, the creation of something that did not exist before, is just about the greatest ideal there is. He sent me to Dr Van Slyke, at the Rockefeller Institute Anybody who knows Dr Van Slyke can understand how a man would become diverted from wishing to be a surgeon to becoming a full-time research person I now work with Dr Vincent Dole at the Institute The problem of shock is not within the realm of our work. Our department is concerned with hypertension, which is just a type of disturbed homeostasis

RAYMOND P. AHLQUIST: I got into pharmacology in a unique way. I went into it because I liked it I am one of the few who deliberately set out to become a pharmacologist As the first graduate student in Dr J M Dille's Department at the University of Washington, I was there even before he came to take it over

My first job was at South Dakota State College There, among other subjects, I taught pharmacology and pharmacognosy, clinical methods, toxicology, and nurses' arithmetic. On the side, I was pharmacologist for the State Experiment Station, which at that time was interested in growing Ephedra, the plant, as a potential source of

ephedrine From a large field I gathered, dried, and ground Ephedra plants. I extracted and purified the ephedrine and tested it in animals. Thus I became interested in the sympathomimetic agents. This project ended when ephedrine was synthesized

Then I went to the Medical College of Georgia under Dr R A Woodbury, where we studied the human uterus and the cause and treatment of dysmenorrhea, which involved the use of ephedrine and other sympathomimetic agents Since 1947, I have been interested in all of the actions of epinephrine and related compounds on pressure, flow, smooth muscle, and other structures and functions At this Conference, I am prepared to defend the good name of epinephrine

GORDON E. W. WOLSTENHOLME: As Director of the Ciba Foundation in London, I am interested in conference techniques Through the kindness of Dr Fremont-Smith, I am here more to observe the mechanics of the meeting than the mechanics of the circulation

I have one slight excuse for being present on this particular occasion. Several of you have mentioned how the war forced your researches in the direction of the subject of this meeting, and to some extent that was true for me, too I was put in charge of a British blood transfusion unit in the Mediterranean, and was in that work for altogether five years I had people of British, and some eleven other nationalities, coming to my unit for training in resuscitation, and I became acutely aware of my ignorance in this field I hope now that you are all about to enlighten me

JEAN OLIVER. I am a pathologist who has been interested for many years in the structure and function not of that Platonic abstraction, the kidney, but of the nephrons, which are organs which we have isolated, manipulated, and, to take up Dr Knisely's dare, even measured in all their constituent parts during various states of health and disease. Since these structures are seriously affected by the general disturbances of shock, that explains my presence here

R E. HAIST· I am interested in experimental shock, particularly in the metabolic aspects of shock As a graduate student, I came under the stimulating influence of Charles Best, so that it is not strange that I developed an interest in

diabetes and carbohydrate metabolism. That has been my main interest for the last fourteen years I was asked to work on shock, and, as quickly as I could, I turned to the metabolic aspects of the problem

F. DOUGLAS LAWRASON: I was in the very happy but somewhat anomalous state of being a hematologist studying electrolyte and renal physiology at Yale when, in 1950, Dr. M C Winternitz convinced me that there was a need for the National Research Council to aid in the coordination of research in the field of blood The great problem was, and continues to be, the preservation of the red cell I found myself closely associated with groups of investigators studying shock, particularly the plasma volume expanders, and I became interested in the metabolic aspects and the circulatory dynamics of shock

ROBERT CLARKE· I was asked to go to the Army Medical Service Graduate School at Walter Reed about two years ago, from the Army Laboratory at Fort Knox where I was studying kidney physiology, having been introduced to that subject by Dr Homer Smith, as Dr. Selkurt was I was given the opportunity to organize a group of young medical officers for studies of the kind of shock which the Army was particularly interested in because of its experience in Korea I now have several young men whose feet I am attempting to keep on the ground, and whose heads I am still hoping to keep in the clouds

JACOB FINE Until World War II, I had a special interest in the pathologic physiology of intestinal obstruction Shock developing in intestinal obstruction was one of the popular subjects of discussion among surgeons When the war started and interest in shock rose, I felt we might understand intestinal obstruction better by studying shock I reread Cannon's monograph published in 1923,* but thought it was a little dated Then I got hold of an up-to-date book, published in several editions The definition of shock changed with each edition and sounded all too pat Incurable shock, the book said, was largely a case of leaking capillaries, but the evidence was scant and dubious, so my laboratory began by testing this hypothesis. We long ago abandoned that theory, and our interest is now concerned with the cause of unresponsiveness to transfusion

*Cannon, W B *Traumatic Shock* London and New York, Appleton, 1923

U. S. von EULER: My interest in shock really began when Dr. Shorr sent me the kind invitation letter to this Conference, although perhaps there was some latent interest in it before I began to get interested in active substances occurring in the body when working with Sir Henry Dale at the National Institute of Medical Research in London, in 1930 Dr. Gaddum and I had an opportunity to see that the active substance in extracts of the intestine which caused increased motility in an isolated intestine was not only acetylcholine, as was generally thought at that time, but that there was also some other active substance, later mostly referred to as substance P, which I don't suppose many have heard of However, it showed that tissues and organs could contain specific and active substances. That stimulated my interest, and I slowly went forward to look for substances with sympathomimetic action

ALAN C. BURTON. I served physics for nine years, and it was, I suppose, just an accident that I became interested in physiology The physics problem that I had been given was: Why did people get fever when they were in a high-frequency field? This turned out to be quite easy to solve. The doing of it brought me into contact with doctors who were using high frequency currents to give people artificial fevers, and becoming interested in what the doctors were talking about, it seemed to me that this would be the very field to go into I was quite sure at that time that the lack of progress of biologists and physiologists in their science was entirely due to the fact that they did not know how to apply the beautifully precise and quantitative measurements that we physicists knew about, and that all I had to do was enter the field and in a few years I could clear up the whole science for them.

I went to Professor Murlin at Rochester, and became extremely interested in human calorimetry and biogenetics, the heat exchange of the animal body, heat production, and so on, and I still have an abiding interest in the fundamental problems of biogenetics But after a couple of years, I began to get a little more humble, and I realized that there was more to this field than I had thought and that it was high time I learned something about the fundamentals of it. I was delighted to have the luck of getting a Rockefeller Training Fellowship and of taking courses in

diabetes and carbohydrate metabolism That has been my main interest for the last fourteen years I was asked to work on shock, and, as quickly as I could, I turned to the metabolic aspects of the problem

F. DOUGLAS LAWRASON. I was in the very happy but somewhat anomalous state of being a hematologist studying electrolyte and renal physiology at Yale when, in 1950, Dr. M C Winternitz convinced me that there was a need for the National Research Council to aid in the coordination of research in the field of blood The great problem was, and continues to be, the preservation of the red cell I found myself closely associated with groups of investigators studying shock, particularly the plasma volume expanders, and I became interested in the metabolic aspects and the circulatory dynamics of shock

ROBERT CLARKE· I was asked to go to the Army Medical Service Graduate School at Walter Reed about two years ago, from the Army Laboratory at Fort Knox where I was studying kidney physiology, having been introduced to that subject by Dr Homer Smith, as Dr Selkurt was I was given the opportunity to organize a group of young medical officers for studies of the kind of shock which the Army was particularly interested in because of its experience in Korea I now have several young men whose feet I am attempting to keep on the ground, and whose heads I am still hoping to keep in the clouds

JACOB FINE. Until World War II, I had a special interest in the pathologic physiology of intestinal obstruction Shock developing in intestinal obstruction was one of the popular subjects of discussion among surgeons When the war started and interest in shock rose, I felt we might understand intestinal obstruction better by studying shock I reread Cannon's monograph published in 1923,* but thought it was a little dated Then I got hold of an up-to-date book, published in several editions The definition of shock changed with each edition and sounded all too pat. Incurable shock, the book said, was largely a case of leaking capillaries, but the evidence was scant and dubious, so my laboratory began by testing this hypothesis We long ago abandoned that theory, and our interest is now concerned with the cause of unresponsiveness to transfusion

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MARCEL GOLDENBERG: Studying the hemodynamic response of man to nor-epinephrine and epinephrine on a team headed by the late Dr Eleanor de F. Baldwin, the results suggested the use of nor-epinephrine in hypotensive states. We tried it first in the shock-like state which often follows thoraco-lumbar sympathectomy, and then started to use it in other types of shock, but the resistance against the use of nor-epinephrine in hemorrhagic and surgical shock was so firm that we had to give it up

FRANK FREMONT-SMITH: My father, who was a physician, and I think a great physician, had a pronounced influence on my life and attitudes. The next great influence was Mr Griswold, my physics teacher who introduced me at the age of sixteen to radioactivity, which at that time and since has always been fascinating to me As a third-year medical student, I heard Lewis Week speaking at the Peter Bent Brigham Hospital on his research on cerebrospinal fluid, and that was a turning point in my life. It led me into the field which occupied my thoughts and energies for many years After I had finished an internship under Francis W. Peabody, I was invited to go to the Massachusetts General Hospital by Dr. James Bourne Ayer, Professor of Neurology at Harvard, to do research on the cerebrospinal fluid My experience when I first went there, I think, is worth recounting I called upon him, and he said, "Come on over. I'll show you the laboratory" He took me over to the laboratory, which was a small room in the pathology laboratory, and he said, "Here it is" Then he turned to leave I said, "But, Dr Ayer, what shall I do?" He said, "Well, do some research," and walked out. That was all the instruction I ever had, but I always had his support, which was wonderful

In 1936, I was asked to come down to be looked over by the trustees of the Macy Foundation to see whether they would like to have me come into the Foundation. The first of the trustees that I had to see was Dr Stewart Paton, who was a very wonderful gentleman, a neurologist. I went to call on him in his apartment One of the first questions he asked me was, "Dr Fremont-Smith, do you believe in conferences?" I had never given the matter much thought I did not know what the right answer was Should I say, no? I thought, "That can't be right" I

the fundamental medical sciences. Again, I think I was very fortunate in that it was decided to send me to the University of Pennsylvania, where I had the benefit of being under the wing of the late Professor Bazett and the many other distinguished physiologists that were there at that time, and still are. I decided that I would try to convince people I was a physiologist, and keep quiet about the physics. It was a wise one, I think, because I found that biophysicists had a very bad reputation; some of them still have. There were lots of physicists that just blundered into working with biological material. They used x-rays on seedlings, let us say, and wrote papers about it. The papers were useless because they were quite ignorant of the fundamental properties of the material they were working with. It seemed to me not only bad biology and physiology, but bad science, that a scientist should presume to work on something without knowing everything about it that he could master. I stuck to that course, and people began to let me into physiological societies. My interest in temperature regulation increased, and, of course, that led me inevitably to be interested in the peripheral circulation and how it played its part in temperature regulation.

Then came the war, and, like most of you, I was put to work on very practical problems, such as heat exchanges, protection from the cold, and so on. At the end of the war, I was so thoroughly fed up with doing so-called useful things that I decided I would look around for something that would appear to be quite useless in research, and as a special interest, I started working on the fundamental physics of small blood vessels. I have been engrossed in that ever since.

SILVIO BAEZ: After completing a year of postgraduate work in physiology at Cornell in 1945, I was fortunate enough to become a member of Dr. Shorr's group for the study of factors regulating peripheral vascular homeostasis in health and disease. I don't recall being told to study medicine, and perhaps I would have chosen to become a flyer in the Air Corps, but three years of waging war in an infantry outfit seem to have oriented my interest toward the study of human behavior in general, and biology in particular.

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quickly added, "I do, if they last long enough." I think that was a prophetic statement, because I have been trying to make them last long enough ever since

EPHRAIM SHORR: As a student at Yale, I early came under the influence of Lafayette Mendel In order to graduate, I had to conform to the practice of writing a thesis, and as I had some interest in ductless glands, I decided to work with Dr. Sam Harvey. We had at Yale, at that time, a great deal of laboratory space, so that as a medical student I enjoyed for three years the possession of a laboratory and a budget, and in addition to that some spare time, which is unusual these days. I elected to work on the relationship of the adrenals to cholesterol metabolism, and in the course of these studies I did not discover, although the evidence was right under my eyes, the basis for the lethal effects of adrenalectomy. I had been working with Underhill, who had taught me when studying blood constituents, to take into account any hemoconcentration or hemodilution Thus, I carefully corrected all my figures for the hemoconcentration which was occurring in devastating fashion after adrenalectomy of the animal, meanwhile disregarding the fact that a marked reduction in blood volume was taking place (I might say that I am still working on the adrenals, though not in particular relationship to cholesterol metabolism)

In Dr. Underhill's laboratory, I worked on all types of problems, along with a half dozen other students One evening we received a call to meet him at the hospital When we arrived there he said, "There has been a fire at the Roger Sherman Theatre There are thirty severely burned patients, and we are now going to carry out the first experiment in keeping up blood volume in humans in burn shock" For three weeks, every four hours, we did all kinds of studies on these patients, maintaining the blood volume with saline That was the manner of my early introduction to shock

After my internship, Dr Mendel suggested that I get some quantitative experience in medicine, and I then spent a year with Dr Eugene Dubois I was offered the only job available, that of a technician analyzing urines, bloods, stools, and what not I took this, and in addition worked with the calorimeter I became interested in the

work of Meyerhof and Warburg on intermediary metabolism via respiration studies which was emerging at the time That I did for many years, wandering into all sorts of fields, keeping close to the clinic at the same time During the second World War, I was stimulated by the first Macy shock meeting, to give some consideration to the metabolic aspects of shock Dr Furchgott, with whom I was fortunate enough to be associated at the time, began to study the defects which might occur in a variety of tissues during shock, and particularly the effect of anoxia upon isolated smooth muscles We were stimulated by the work of Chambers and Zweifach, which indicated that the fatal set of events might be greatly influenced by the behavior of the terminal vascular beds After a time the Josiah Macy, Jr Foundation made it possible for Dr. Zweifach to join us in an attempt to relate the altered behavior of the musculature of the terminal vascular bed to metabolic products that might arise in the course of the shock syndrome Since the end of the war, Dr Mazur, a biochemist, and Dr Baez, a physiologist, have been part of our team, enabling us to proceed on a multidiscipline level

METABOLIC ASPECTS OF HEMORRHAGIC AND TRAUMATIC SHOCK

FRANK L. ENGEL
Department of Medicine and Physiology
Duke University School of Medicine

When Dr Shorr asked me to discuss this subject I hesitated on the ground that I did not feel that I had very much of anything new to contribute However, I finally agreed, more or less with the idea that although much of what I am going to present is old material, going back as far as ten years ago, it might perhaps be of some use to determine if the old wine can be put into new bottles, i e , to see whether these old data can be reinterpreted in light of what we have learned about shock in the last ten years

Much of the work* that I shall speak about began at Yale University, and in order to avoid repeating each time what each individual's contribution was, I might mention now who was involved As I told earlier, it started more or less with my accidental observations on bleeding, and thereafter many people in the Department of Physiological Chemistry (Dr C N H Long, Chairman) were involved, notably Drs Alfred Wilhelmi, Jane Russell, Mary Winton, Helen Harrison, George Sayers, Henry Harkins, Walter Burdette, Mrs Marion Sayers, and Mrs Mildred Engel Most of this group have not carried out additional studies on shock, except for Dr Burdette, who has continued work on shock at Louisiana State, and for ourselves at Duke

I propose to consider the metabolic response to injury and shock, first in terms of the earliest, and perhaps relatively nonspecific changes, and going on to sketch in the total picture of the metabolic changes that are fairly familiar as occurring during middle and late shock The evidence for differential involvement of organs and tissues in the course of shock and their significance will be considered, and the effects of procedures such as conditioning and

*Certain portions of the research described in this discussion have been done under contract No DA-49-007-MD-134 with the Medical Research and Development Board, Office of the Surgeon General, Department of the Army

the various types of treatment on the metabolic and circulatory changes, will be evaluated.

As with all other phases of shock, the work on metabolism has been associated with considerable disagreement about what changes take place at what time, and how important any given change is. Much of the disagreement, in my opinion, is rather beside the point, it is mostly a matter of various investigators dealing with different types of experimental situations and making measurements at different times When the available data from such experiments are lined up, they fit together surprisingly well Various species respond a bit differently, according to their innate metabolic characteristics By and large, in the discussion that is going to follow, my remarks will be concerned largely with experiments with rats Where other species have shown similar changes, I hope I shall remember to mention them.

In their approach to metabolic changes in shock, different investigators have had different motives and objectives In our own case, it was the rather simple one of being interested in some of the parameters in metabolism during the reaction to injury and the subsequent development of shock, without having, initially at least, too much of an ax to grind as to whether any one metabolic change is more important than another or as to whether effects on any one organ are necessarily more vital I hope that when the metabolic pattern has been outlined as completely as possible, it will be possible to discern patterns which will indicate what metabolic alterations may prove critical

We did concentrate on one organ, the liver, mostly because of the metabolic lability of that organ Other individuals have approached the problem by focusing on one or another organ as the preliminary source of difficulty, either in terms of metabolic failure of that organ, in a general sense, or in terms of a loss of ability of that organ to detoxify, we shall say, some substance which has a deleterious effect on the circulation Still others have approached the metabolic problem from the standpoint of identifying some toxic material from damaged or hypoxic tissue which might be considered responsible for the perpetuation of the shock state Finally, others have been interested in the problem of treatment and such phenomena as conditioning in terms of whether these are effective on the metabolic or some other level

Knisely When you say "conditioning," do you mean Pavlov's conditioning ?

Engel No, I refer particularly to the process with the Noble-Collip drum whereby animals were subjected to controlled injury, being conditioned in a graded fashion, by tumbling in the drum, to receive a greater and greater amount of injury, until they became resistant to the drum trauma, and also to other types of shock-inducing procedures

Let us consider first the sequence of events in response to hemorrhage or trauma leading to shock We can divide this, more or less arbitrarily, into three phases, the first being the initial response to injury, involving a number of metabolic changes which are nonspecific in the sense that they are relatively independent of the type of injury, although certain changes which are specific to any given type of injury also occur This particular phase of the reaction of the body has been studied in considerable detail in recent years, and we now know a moderate amount about it

There comes next a large no-man's-land between this initial response and the late stage when fairly characteristic changes in metabolism, circulation, and so forth, take place Perhaps the most important phase is the one we know least about, namely, the great area between the initial response to injury and the terminal decompensation Somewhere in here something happens which converts this condition from one which readily responds to blood and plasma to one which goes on relentlessly to death despite fluid replacement, the third stage, or so-called irreversible shock

THE INITIAL RESPONSE TO INJURY

To consider the first of these periods from the metabolic standpoint, there has been tremendous emphasis in the last ten or fifteen years on the role of certain endocrine glands, particularly the adrenal cortex, in the initial response to injury, but I think a lot of misconceptions have grown out of the original concepts of the alarm reaction and the general adaptation syndrome as outlined by Selye

Some of the well-known reactions to injury in the normal organism are listed below

Metabolic Changes

1. Negative nitrogen balance
2. Impaired carbohydrate utilization
3. Fatty liver and tendency to ketosis
4. Negative K^+ and $PO_4^{\equiv}$ balance
5. Na^+, Cl^- and H_2O retention by kidney
6. Hypochloremic, hypokalemic alkalosis

Morphologic Changes

1 Lymphoid tissue and thymus hypoplasia
2 Lymphopenia and eosinopenia
3 Leukocytosis

Endocrine Changes

1 Adrenal hypertrophy
2 Ascorbic acid and cholesterol depletion of adrenal cortex
3 Increase in corticoids in blood and urine

A change in nitrogen metabolism, with a negative nitrogen balance, is the best known response to injury However, even before this is detectable, an alteration in the level of plasma amino nitrogen occurs This takes place after almost any sort of injury or stress and is characterized by a fall in plasma amino acids It occurs in hypophysectomized, adrenalectomized, and adrenomedullated animals, in contrast to the negative nitrogen balance which is dependent on an intact pituitary-adrenal system Although epinephrine, insulin, and growth hormone will cause a comparable fall in amino acids, we cannot attribute the change after injury to an influence of the endocrine glands Its precise significance remains obscure, and it deserves further investigation

Secondly, there may develop varying degrees of impairment of carbohydrate utilization, the so-called traumatic diabetes that was described many years ago Thirdly, there is a tendency to development of fatty liver and ketosis Finally, there occur negative potassium and phosphorus balances, sodium, chloride, and water retention by the kidney, and occasionally hypochloremic, hypokalemic alkalosis if potassium depletion has taken place

Nickerson In connection with your second point, is there a clear impairment of carbohydrate utilization or is a factor of increased glucose production involved?

Engel We cannot answer that accurately Probably, it would be more accurate to speak of carbohydrate tolerance rather than carbohydrate utilization I suspect the change is mostly utilization, but I do not think the point has ever been proved

Loewi Does this occur in the very beginning?

Engel Quite early.

Loewi Does it develop quickly?

Engel The electrolyte changes take some time to develop, but the fall in amino nitrogen, the increase in nitrogen metabolism, the impaired carbohydrate tolerance, the fatty liver, and ketosis certainly can be demonstrated within four hours after certain

injuries. I do not think the others have been studied accurately enough to know whether they occur that early or not

von Euler Do these changes occur in the demedullated animal?

Engel· All will occur in the demedullated animal with the exception of one which I did not include, namely, the rise in blood sugar immediately after injury. This appears to be largely a response of the adrenal medulla

Knisely What is the shortest period of time from injury until one of these changes can be detected? What is the most sensitive indicator in time and in change?

Engel. The earliest one that we have measured is the change in nitrogen metabolism, particularly the fall in amino acid nitrogen. We can detect this within an hour of the introduction of an injury. Of course, the blood sugar rise in response to adrenal medullary secretion occurs early, too

Haist Would you include tourniquet application as a type of injury, or do you exclude that from this discussion?

Engel Yes, I would, but there are many gradations of injuries. The full response will occur with a fairly strong injury, it may not occur with a lesser one, although it takes extraordinarily little stress to cause a fall in the plasma amino nitrogen I have never looked for it in tourniquet injury, but with every other stress that I have examined it occurs, so I suspect it would be found there, too

Haist In some experiments, we observed a rise in amino nitrogen, starting from the time of release of the clamps

Engel Did you measure it before release of the clamps?

Haist Yes It fell somewhat during the period of clamp application and then began to rise as soon as the clamps were removed

Engel I would suspect that the fall with clamp application was the initial response to the injury In response to injury, there are morphologic changes in lymphoid tissue, and eosinopenia as well as endocrine changes These changes include adrenal hypertrophy, ascorbic acid and cholesterol depletion of the adrenal cortex, and an increased amount of corticoids in the blood and urine Since certain of these metabolic changes have been observed with overdoses of adrenal hormone, many investigators have jumped to the conclusion that all these phenomena result from the effects of hypersecretion of adrenal hormone in response to injury

The main point that I wish to make is that this is not the case The adrenal cortex is involved in some way in shock The adrenalectomized animal is extraordinarily sensitive to any traumatic procedure and will readily go into circulatory collapse At the same time, we also know that individuals who may have apparently

normal endocrine function but who are chronically ill, malnourished, or depleted in protein in some way, are somewhat more prone to develop shock when injured than are normal individuals

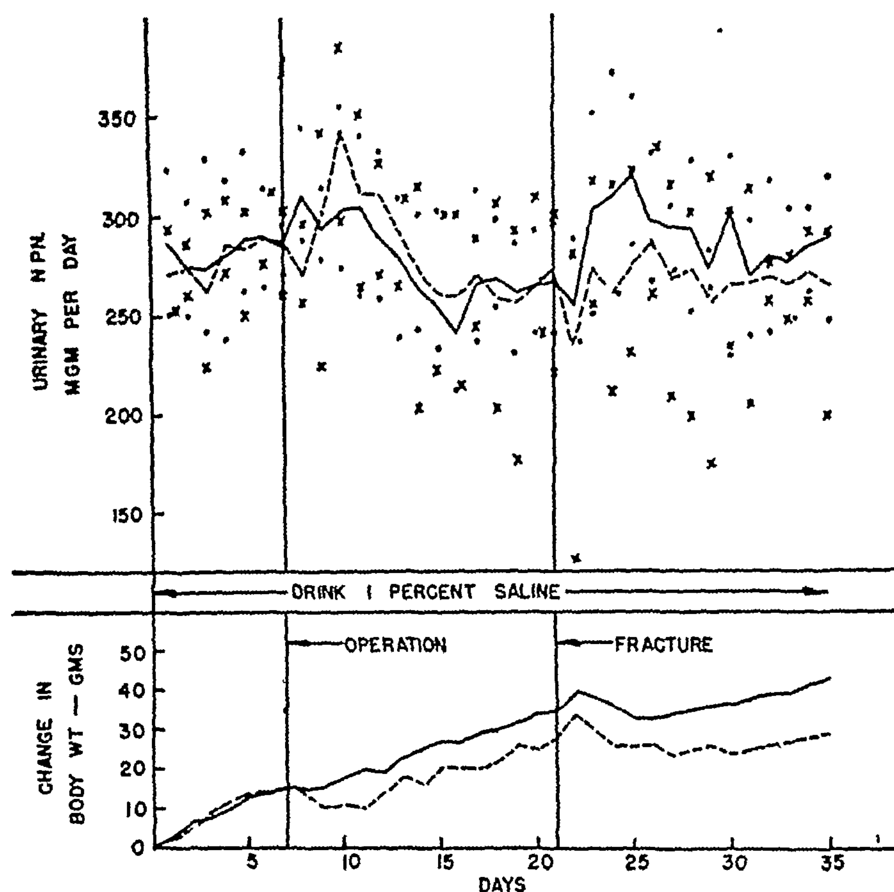


FIGURE 1 The effect of operation and of fractures on the urinary non-protein nitrogen of six pairs of sham-operated (———) and saline-treated adrenalectomized (x - - - -) rats Averages and range of values Reprinted, by permission, from Ingle, D J, Ward, E O, and Kuizenga, M H The relationship of the adrenal glands to changes in urinary non-protein nitrogen following multiple fractures in the force-fed rat. *Am J Physiol* **149**, *510 (1947)*

The first data I should like to present as evidence against the idea that the adrenal cortical hormones themselves are responsible for these changes come from the work of Dwight Ingle, in which he has demonstrated the so-called permissive action of the adrenal hormone in response to fracture (1). Ingle studied nitrogen excretion in normal and adrenalectomized animals after leg fracture, and demonstrated that in normal animals there is an increase in nitrogen excretion, while in adrenalectomized animals, which were maintained on saline, there was no such change (Figure 1) Of course, it had been well known even before this that the adrenalectomized animal does not show the same response to trauma that the normal animal

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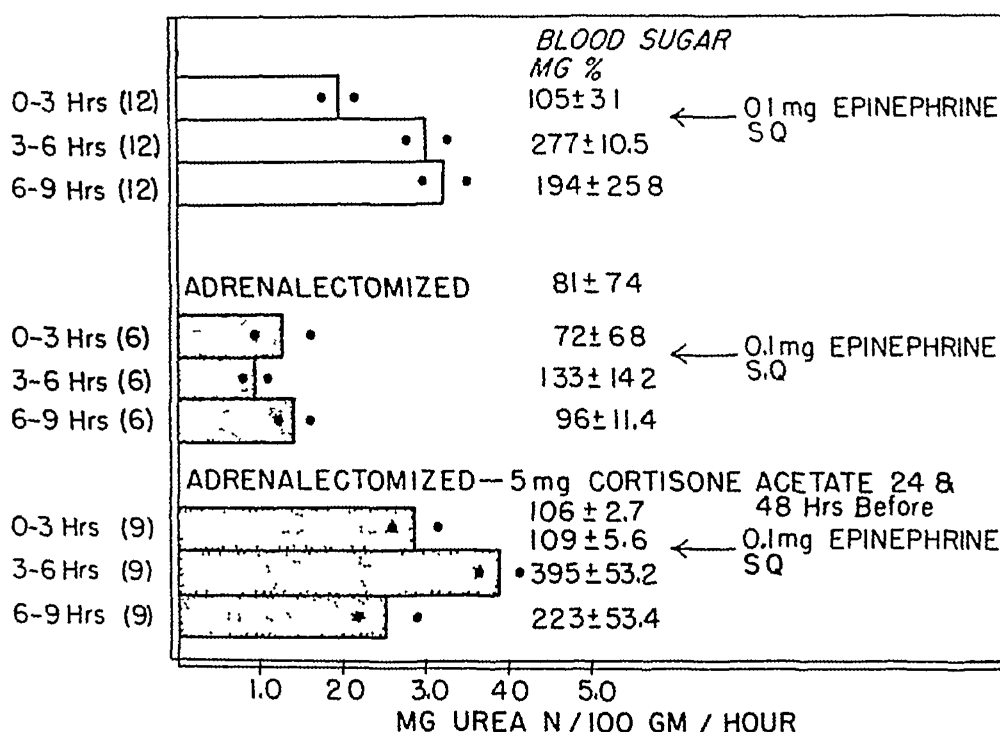


FIGURE 3 Effect of epinephrine on urea formation of nephrectomized and adrenalectomized-nephrectomized rats Horizontal bars represent mean rates of urea nitrogen formation expressed as mg urea N/100 gm /hr In this and subsequent Figures dots indicate ± one standard error Epinephrine was injected subcutaneously at the end of a 3-hour control period Blood sugar was measured at 3-hour intervals Note sustained increase in urea formation after epinephrine in control nephrectomized rats (white bars), no response in adrenalectomized rats, and temporary increase in cortisone-maintained adrenalectomized rats Reprinted, by permission, from Engel, F L A consideration of the roles of the adrenal cortex and stress in the regulation of protein metabolism *Rec Progr Hormone Res* 6, 277 (1951)

Figure 3 brings out one additional point. that a continued increase in adrenal hormone, as suggested by Ingle, is necessary to sustain this response In this study, we measured the rate of urea accumulation in nephrectomized rats as a measure of changes in nitrogen metabolism in response to injury and in response to hormone action The technique was to nephrectomize animals 15 hours before the experimental procedure and to measure the rate of rise of urea in the blood at one- to three-hour intervals before and after various procedures The total amount of urea was calculated on the assumption that it was equally distributed throughout the body water, which was taken as 63 per cent of the body weight. A large dose of epinephrine was used as the stressing agent, a dose very much in excess of that used by Long to stimulate the pituitary adrenal axis It was not intended to produce that sort of response but to demonstrate that epinephrine can trip off the metabolic response of trauma As shown in Figure 3, in the normal animal,

does, it readily succumbs, usually in hypoglycemia When the adrenalectomized animals were maintained on a constant dose of cortical hormone and the same injury induced, there was exactly the same response in nitrogen excretion in the adrenalectomized animal as in the normal animal (Figure 2). In the adrenalectomized rats receiving adrenal extract, the adrenal hormone was a constant factor, and hence the change in nitrogen excretion cannot be attributed to a hypersecretion of adrenal hormone by the gland.

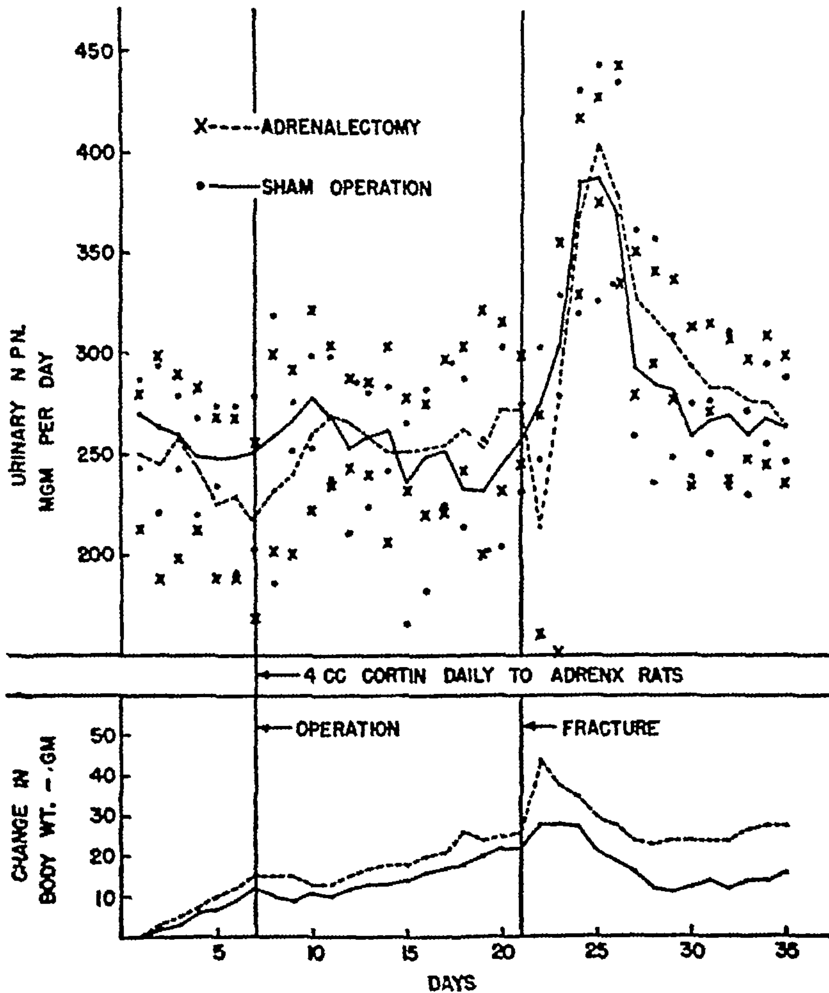


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In later work, Ingle has shown the same response with respect to sodium chloride retention and a number of other phenomena (2). We have confirmed his findings (3,4)

Engel I cannot quote any reference to experimental data which proves that urea is freely diffusible, but I do think there is good evidence that this is true

The only criticism of this particular technique that has been offered was made by Dr Baird Hastings a year or so ago About 10 per cent (I am not sure of the exact figure. it may be somewhat less) of the urea that is formed by the liver is formed twice In other words, that urea which diffuses from the blood into the gastrointestinal tract is acted upon by bacteria which have urease in them deep in the crypts of the intestine The urea is converted there to CO_2 and ammonia, and the ammonia is then reabsorbed into the portal vein and is re-formed into urea

Green. Most of the membranes are rather freely permeable, are they not? The only one I know of that is not permeable to urea is the gill membrane in the elasmobranch fishes, as described by Dr Homer W Smith (6)

Birch I am aware of this, but I just wonder whether or not, when one is observing one compartment for a sampling of urea, some peculiarities in the distribution of urea might develop, without one's knowledge, that would not adhere to the general assumptions made in the study Has anyone made an attempt to measure the concentration-time course of urea inside cells simultaneously with measurement of the concentration-time course in the blood?

Oliver Didn't Marshall (7) examine that point years ago and find that the urea is, to all intents and purposes, evenly distributed throughout the body except in the nervous tissue?

Engel It was my impression that that was so, although I didn't know who first demonstrated it

Loewi One cannot, in my opinion, compare the behavior of urea, which like all water- and lipoid-soluble substances is freely diffusible with nonlipoid-soluble substances like potassium and sodium salts, which penetrate cells by a process which is extremely slow in comparison with that of free diffusion

Moe I wonder whether the adrenal cortex is essential for the increase in the over-all metabolic rate which is produced by epinephrine Is this a general phenomenon, or is this a nitrogen phenomenon?

Engel So far as I know, the adrenal cortex is not essential for that response to epinephrine This brings us to the next point I think most people are now in agreement with Ingle's concept of the permissive action of the adrenal cortex We have extended this concept to include evidence that the presence of the adrenal hor-

epinephrine stimulated a change in nitrogen metabolism in three hours When the same dose of epinephrine was given to the adrenalectomized animal, there was no change in the nitrogen metabolism. When epinephrine was injected into the adrenalectomized animal, maintained on a crystalline suspension of cortisone in saline which forms a depot, there was a prompt increase in nitrogen metabolism, just as in the normal animal, but it was sustained for only three hours Our interpretation has been that the amount of steroid circulating from this crystalline depot was not sufficient to maintain the response longer than three hours Ingle has recently done a number of experiments with adrenalectomized-depancreatized rats subjected to stress which tend to support this conclusion (5).

From these experiments, there is good evidence that the adrenal cortex is necessary to, but not responsible for, the metabolic response to injury and that a sustained secretion of adrenal hormone is necessary to keep up the reaction

Nickerson Do the data represent the total amount of urea present, or the rate of formation?

Engel· These are rates of formation. The blood urea is measured and then the amount of urea that has been formed in the three-hour period is calculated by the difference from the control urea nitrogen level, assuming the urea to be equally distributed throughout the body water, which is taken as 63 per cent of body weight. The results are expressed as milligrams urea nitrogen per 100 gm per hour

Burch: Do you think that the distribution of the urea might change, that it might be uneven and in a distribution different from that generally accepted as correct?

Engel Urea is so freely diffusible that I think there would be little worry about that

Burch· Electrolytes, for example, may change their distribution under the influence of varying normal and abnormal physiologic states.

Engel Electrolytes are diffusible but they distribute themselves quite differently than does urea

Burch I was wondering whether there was any evidence to show that urea might, in certain circumstances, behave differently under the influence of different hormones. Since you are simply examining one compartment containing urea, the intracellular distribution might be altered without reflection in the extracellular-sampled compartment.

Engel I cannot quote any reference to experimental data which proves that urea is freely diffusible, but I do think there is good evidence that this is true

The only criticism of this particular technique that has been offered was made by Dr Baird Hastings a year or so ago About 10 per cent (I am not sure of the exact figure. it may be somewhat less) of the urea that is formed by the liver is formed twice In other words, that urea which diffuses from the blood into the gastrointestinal tract is acted upon by bacteria which have urease in them deep in the crypts of the intestine The urea is converted there to CO_2 and ammonia, and the ammonia is then reabsorbed into the portal vein and is re-formed into urea

Green Most of the membranes are rather freely permeable, are they not? The only one I know of that is not permeable to urea is the gill membrane in the elasmobranch fishes, as described by Dr Homer W Smith (6).

Burch. I am aware of this, but I just wonder whether or not, when one is observing one compartment for a sampling of urea, some peculiarities in the distribution of urea might develop, without one's knowledge, that would not adhere to the general assumptions made in the study Has anyone made an attempt to measure the concentration-time course of urea inside cells simultaneously with measurement of the concentration-time course in the blood?

Oliver Didn't Marshall (7) examine that point years ago and find that the urea is, to all intents and purposes, evenly distributed throughout the body except in the nervous tissue?

Engel It was my impression that that was so, although I didn't know who first demonstrated it

Loewi One cannot, in my opinion, compare the behavior of urea, which like all water- and lipoid-soluble substances is freely diffusible with nonlipoid-soluble substances like potassium and sodium salts, which penetrate cells by a process which is extremely slow in comparison with that of free diffusion

Moe I wonder whether the adrenal cortex is essential for the increase in the over-all metabolic rate which is produced by epinephrine Is this a general phenomenon, or is this a nitrogen phenomenon?

Engel So far as I know, the adrenal cortex is not essential for that response to epinephrine This brings us to the next point I think most people are now in agreement with Ingle's concept of the permissive action of the adrenal cortex We have extended this concept to include evidence that the presence of the adrenal hor-

epinephrine stimulated a change in nitrogen metabolism in three hours When the same dose of epinephrine was given to the adrenalectomized animal, there was no change in the nitrogen metabolism When epinephrine was injected into the adrenalectomized animal, maintained on a crystalline suspension of cortisone in saline which forms a depot, there was a prompt increase in nitrogen metabolism, just as in the normal animal, but it was sustained for only three hours Our interpretation has been that the amount of steroid circulating from this crystalline depot was not sufficient to maintain the response longer than three hours Ingle has recently done a number of experiments with adrenalectomized-depancreatized rats subjected to stress which tend to support this conclusion (5)

From these experiments, there is good evidence that the adrenal cortex is necessary to, but not responsible for, the metabolic response to injury and that a sustained secretion of adrenal hormone is necessary to keep up the reaction

Nickerson Do the data represent the total amount of urea present, or the rate of formation?

Engel These are rates of formation The blood urea is measured and then the amount of urea that has been formed in the three-hour period is calculated by the difference from the control urea nitrogen level, assuming the urea to be equally distributed throughout the body water, which is taken as 63 per cent of body weight The results are expressed as milligrams urea nitrogen per 100 gm per hour

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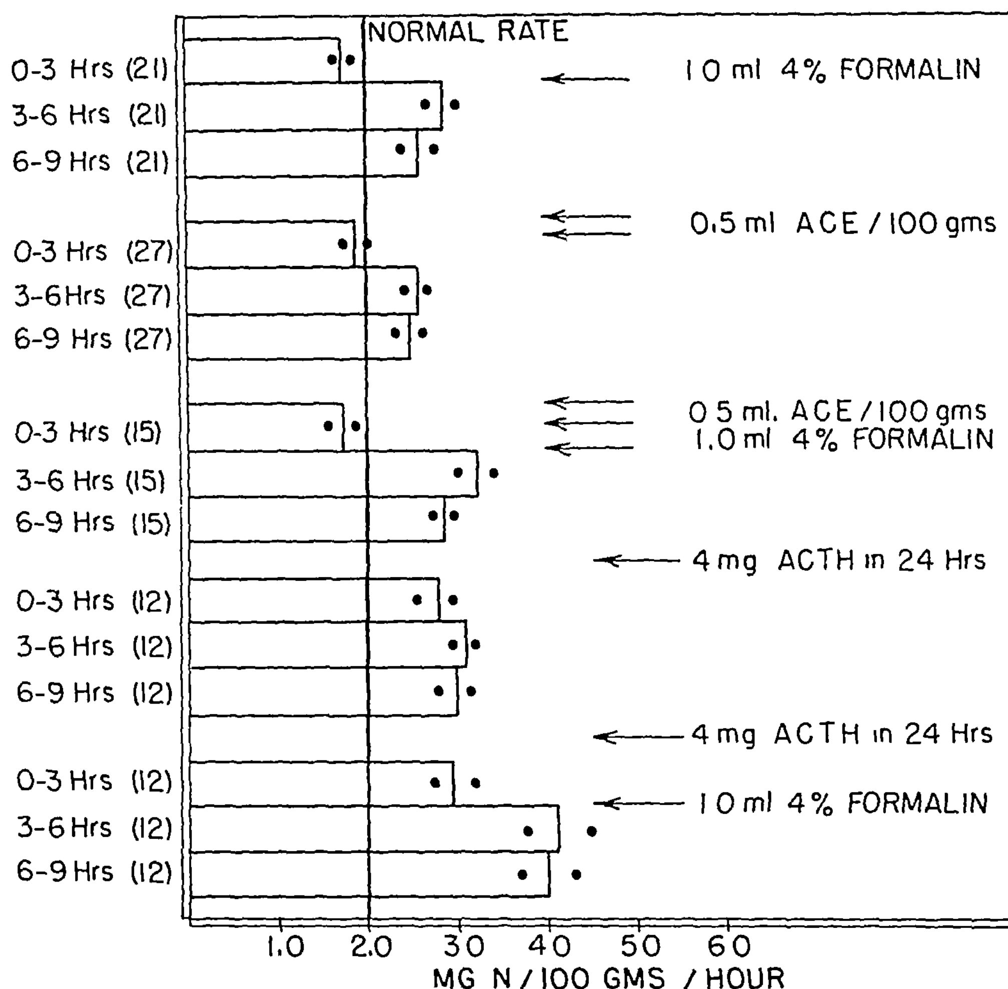


FIGURE 5 Combined effects of formalin stress and ACE and ACTH on urea formation Note immediate response to formalin given at 3 hours compared with delayed response to ACE given at 0 hour Neither immediate pretreatment with ACE or chronic pretreatment with ACTH with consequent increase in nitrogen metabolism modified the magnitude of subsequent response to formalin Reprinted, by permission, from Engel, F L A consideration of the roles of the adrenal cortex and stress in the regulation of protein metabolism *Rec Progr Hormone Res* 6, *277 (1951)*

In contrast to this, as seen in Figure 5, when formalin was injected as a damaging agent, the change in nitrogen metabolism occurred within the next three-hour period This is comparable to that after epinephrine injections (Figure 3).

Green How was the formalin given?

Engel By subcutaneous injection

Shorr These changes were regardless of the dose of adrenal cortical extract?

Engel Yes We have used larger doses of adrenal cortical extract, or ACTH, and not reproduced the immediate change in nitrogen metabolism which is stimulated by these stressful stimuli.

mone seemingly does something to alert the organism to respond metabolically, and perhaps otherwise, to stressful stimuli

Part of our evidence for this independence and interdependence of the adrenal cortex and the stress stimulus came from experiments on the time that it took for different adrenal hormone preparations (adrenal cortical extract, ACTH intraperitoneally, ACTH intravenously by 40 minute infusion, and ACTH by a single injection) to influence urea formation in the nephrectomized rat In every case, we found that it took longer than three hours from the time of injection for a change in nitrogen balance to begin There never was a measurable change in nitrogen metabolism in less than three hours after any preparation of ACTH or adrenal hormone (Figure 4).

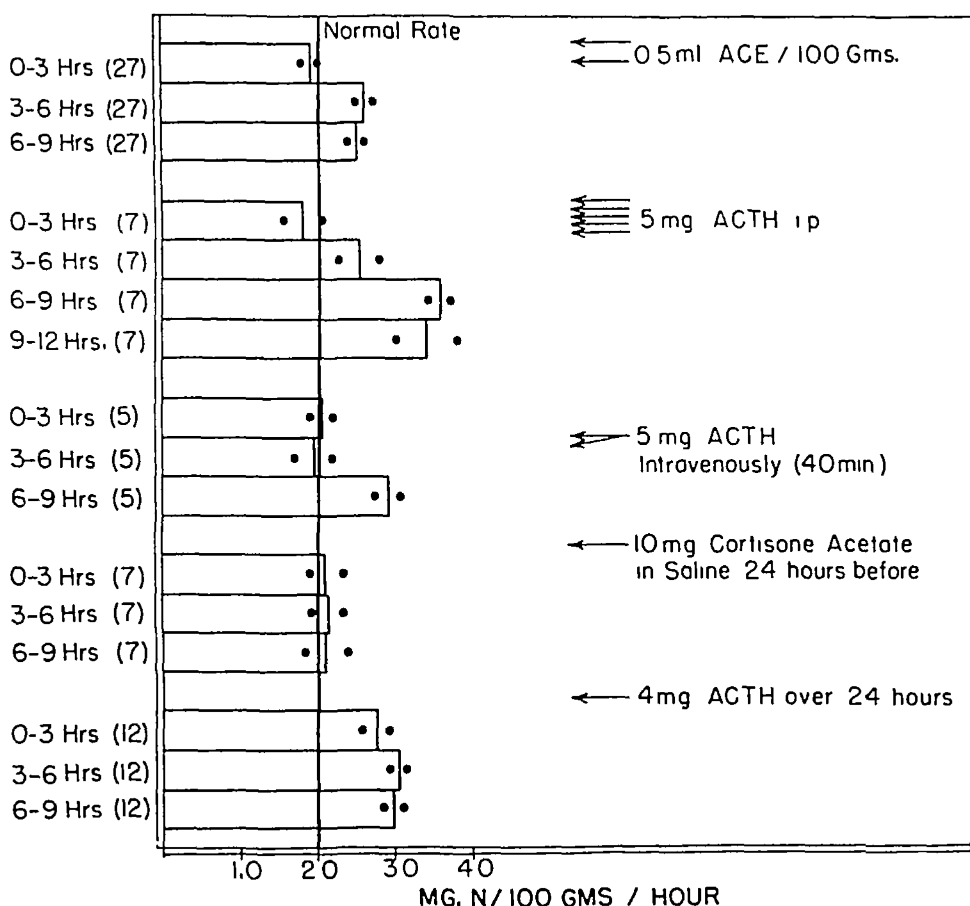


FIGURE 4 Effects on urea formation of adrenal cortical extract injected at 0 and 1 hour, ACTH, 1 mg every 30 minutes intraperitoneally from 0 to 2 5 hours, ACTH, 5 mg intravenously from 3 to 3 hours and 40 minutes, and cortisone and ACTH chronically Note that acute response to ACE or ACTH always occurred 3 or more hours from the beginning of treatment Contrast with immediate response to epinephrine (Figure 3) and formalin (Figure 5) Reprinted, by permission, from Engel, F L A consideration of the roles of the adrenal cortex and stress in the regulation of protein metabolism *Rec Progr Hormone Res* 6, *277 (1951)*

until the adrenal cortex was stimulated and then the response occurred by a permissive action

Moe There is a suggestion of a response to a so-called subthreshold dose. Is the response to stress plus cortical extract more than additive?

Engel The increase after adrenal extract and 0 5 ml formalin is significantly greater than either one alone and may be additive.

Haist What dose of insulin was required to give a similar effect?

Engel Eight hundredths of a unit per one hundred grams, subcutaneously

Haist. I find it difficult to think of the injection of insulin in small doses as a stressful situation.

Engel If insulin is given intraperitoneally instead of subcutaneously, there can be quite an early response, depending, of course, upon how fast the hypoglycemia develops It is the response to the hypoglycemia which is the stressful stimulus, not the insulin itself. With a small dose of insulin subcutaneously and gradual development of hypoglycemia, there may be no change in nitrogen metabolism within the first three hours, but it will appear later on in the period of the animal's recovery from the mild hypoglycemia that occurs But if the adrenal extract is given simultaneously with the insulin and the fall in blood sugar then occurs, the response occurs right after the hypoglycemia

Haist. Wouldn't there be some counteraction of the insulin effect by the extract?

Engel Yes and no In neither rats nor man have we found an appreciable inhibiting effect of ACTH, or adrenal hormone, on insulin hypoglycemia

We have extended this concept to some considerations of the effects of cortical steroids on certain relatively simple metabolic processes in normal, as compared to ill, individuals It has been clearly shown that the administration of cortisone, or ACTH, may cause impairment of the glucose tolerance test, insulin resistance, and so forth, in man Most of the studies along these lines have been done on hospitalized subjects in various stages of illness However, there is no doubt but that these changes occur to some degree even in normal people We were interested in seeing whether we could demonstrate a potentiation by cortisone of the response to injury in man by comparing the responses to glucose and insulin tolerance tests in normal, healthy medical students versus ill people in the hospital, with and without cortisone treatment

Shorr. In other words, you cannot, by increasing the degree of stimulation, reproduce the rate at which the change occurs with formalin.

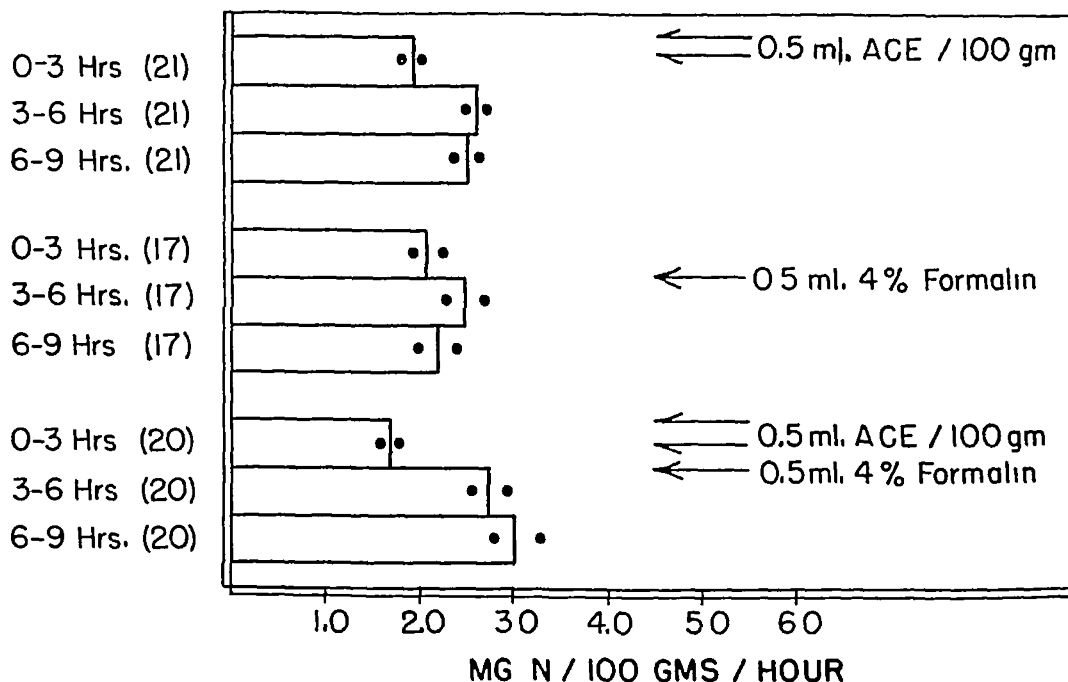


FIGURE 6 Effect of subthreshold stress (0 5 ml formalin) and ACE on urea formation Compared with larger dose of formalin, 0 5 ml formalin subcutaneously caused no stimulation of nitrogen metabolism When given to ACE-treated rats, the subsequent increase in urea formation was signficantly greater than that from either agent alone Reprinted, by permission, from Engel, F L A consideration of the roles of the adrenal cortex and stress in the regulation of protein metabolism *Rec Progr Hormone Res* **6**, *277 (1951)*

Engel That's right This perhaps can be seen better in Figure 6, which brings up a more critical point concerning the use of a stressful stimulus which is subthreshold in the sense that it does not have any measurable effect on nitrogen metabolism (0 5 ml formalin compared to 1 0 ml formalin in the last experiment, or a small dose of insulin subcutaneously) We found with a variety of these so-called subthreshold stresses that, if a dose of adrenal hormone was given before or even with this subthreshold stress, an immediate response in nitrogen metabolism occurred, as compared to no response from the stress alone and a delayed one from adrenal hormone alone In other words, the presence of an excess amount of adrenal hormone at the time injury was induced tripped off this metabolic reaction immediately, whereas, normally, either there was no measurable metabolic response to this grade of stress or there was a lag from the time the injury was imposed

until the adrenal cortex was stimulated and then the response occurred by a permissive action.

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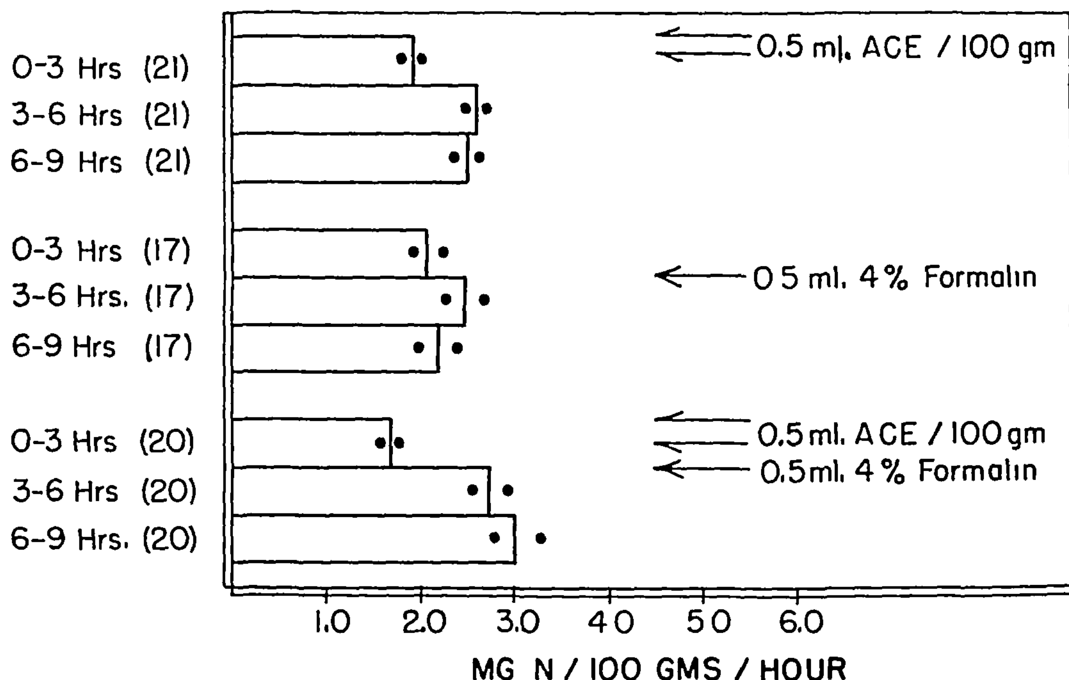


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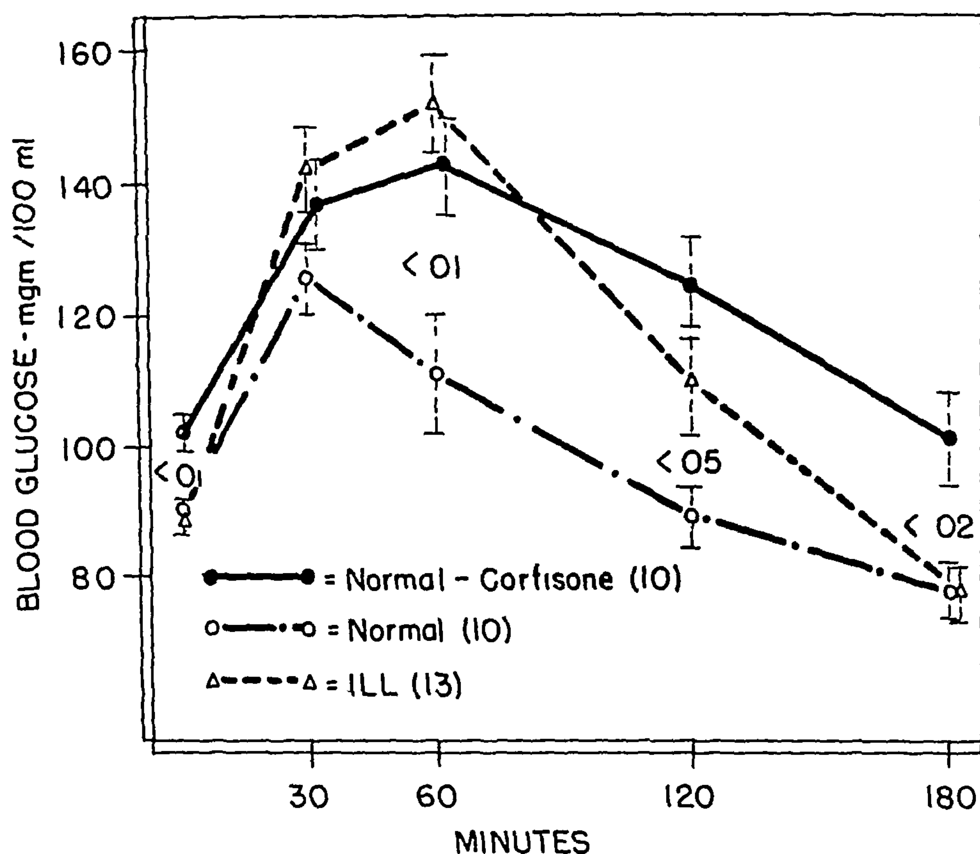


FIGURE 8 Comparative effects of illness and cortisone on the oral glucose tolerance test Note that the response to the two differ by virtue of the fasting and 3-hour levels being significantly higher in the normal subjects receiving cortisone

Figure 8 illustrates data from a group of 13 ill persons on the ward The glucose tolerance test showed the typical impairment that is associated with illness, but it is interesting to point out that it differed from that which is obtained with cortisone (Figure 9) With the latter, there is a significant elevation in the fasting blood sugar to begin with, and the three-hour level is likewise higher than normal In the ill people, the fasting blood sugar starts at the same place as the normal and ends up at the same place, but levels are higher at the 30, 60, and 120 minute points The most important point, however, is that when these ill people were given a single dose of 200 mg of cortisone four hours before the test, there resulted a tremendous impairment of glucose tolerance It might be said that this is because the ill people have a lot of adrenal cortical hormone circulating, and when given 200 mg of cortisone the result is like that in normal subjects given much more hormone The students were given 200 mg of cortisone every day for eight days, and they never showed any greater impairment of glucose

In the first study, ordinary glucose tolerance tests were done on normal medical students with and without previous administration by mouth of 200 mg of cortisone four hours before the test The results of these tests were compared to those in a group of ill subjects in the hospital who were similarly treated The patients represented a cross section of ward patients with organic disease, except that all patients with liver disease, endocrine disorders, diabetes, or a family history of diabetes, were excluded. The students ate in the university dining halls and the composition of their diet was quite close to that offered to the patients. The diet of the latter contained 290 gm. of carbohydrate.

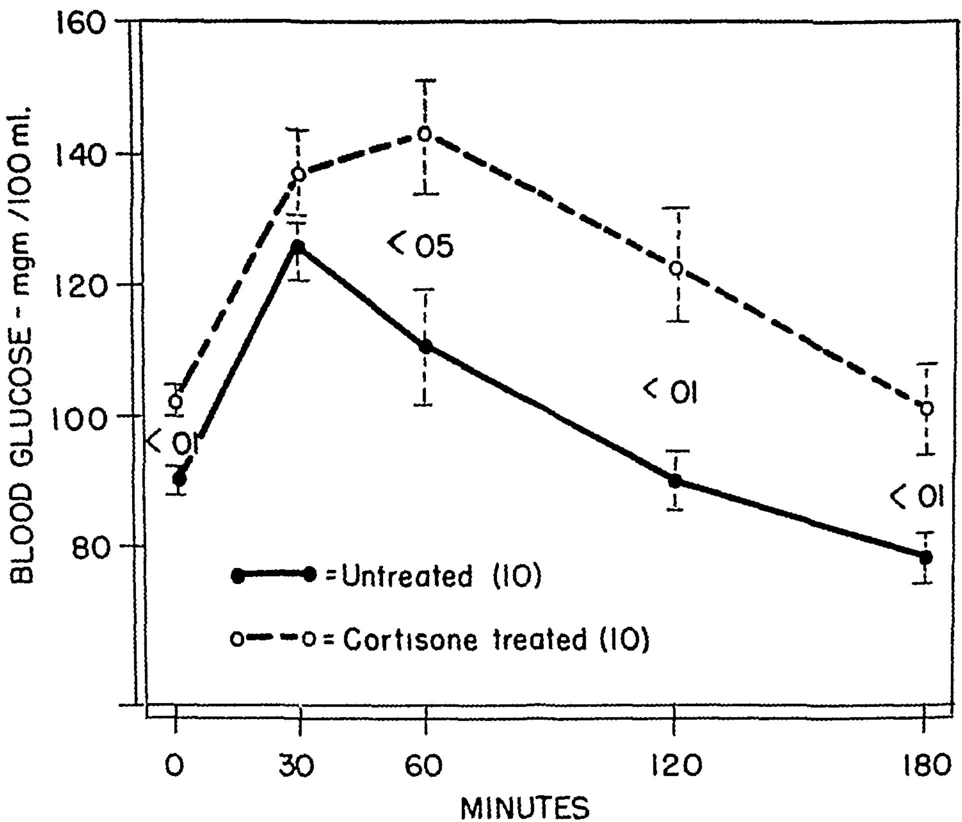


FIGURE 7. Demonstration that a single oral dose of 200 mg of cortisone acetate 4 hours before an oral glucose tolerance test causes a significant increase in blood sugar at all points of the curve except at 30 minutes In this and subsequent figures bars indicate ± one standard error of the mean

Figure 7 represents the normal glucose tolerance test in these students and the glucose tolerance of the same students when given 200 mg of cortisone by mouth four hours before the test You will note that there is a slight but significant impairment of glucose tolerance after cortisone.

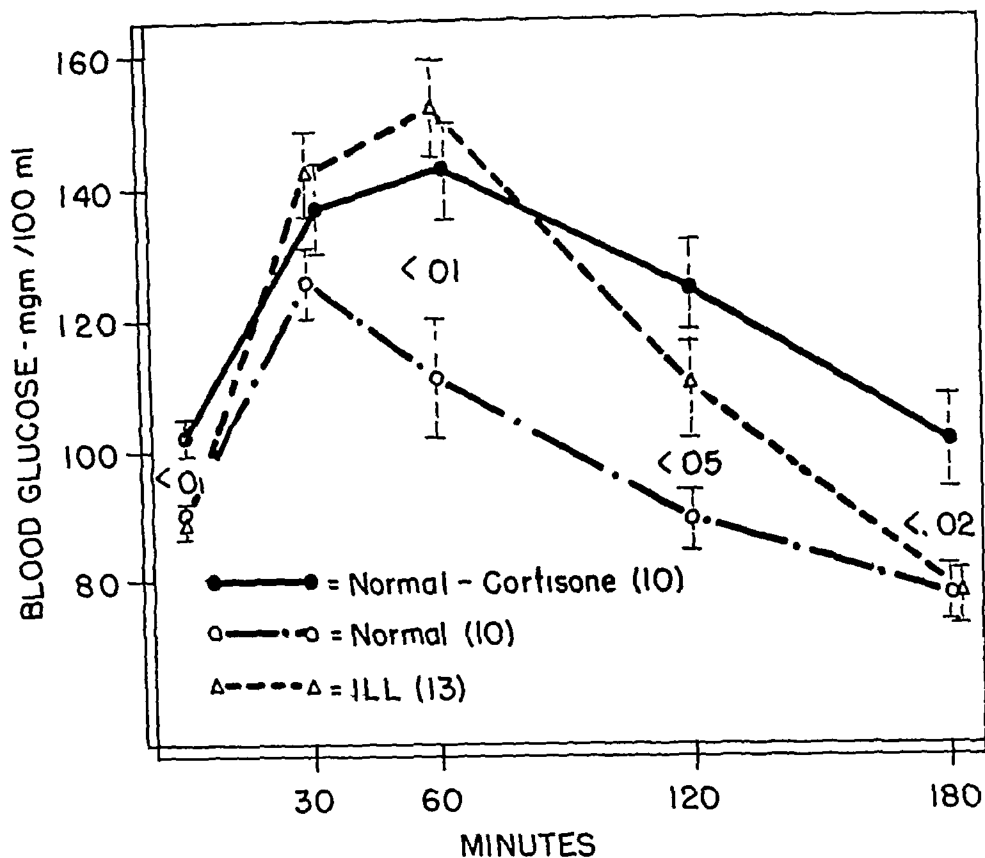


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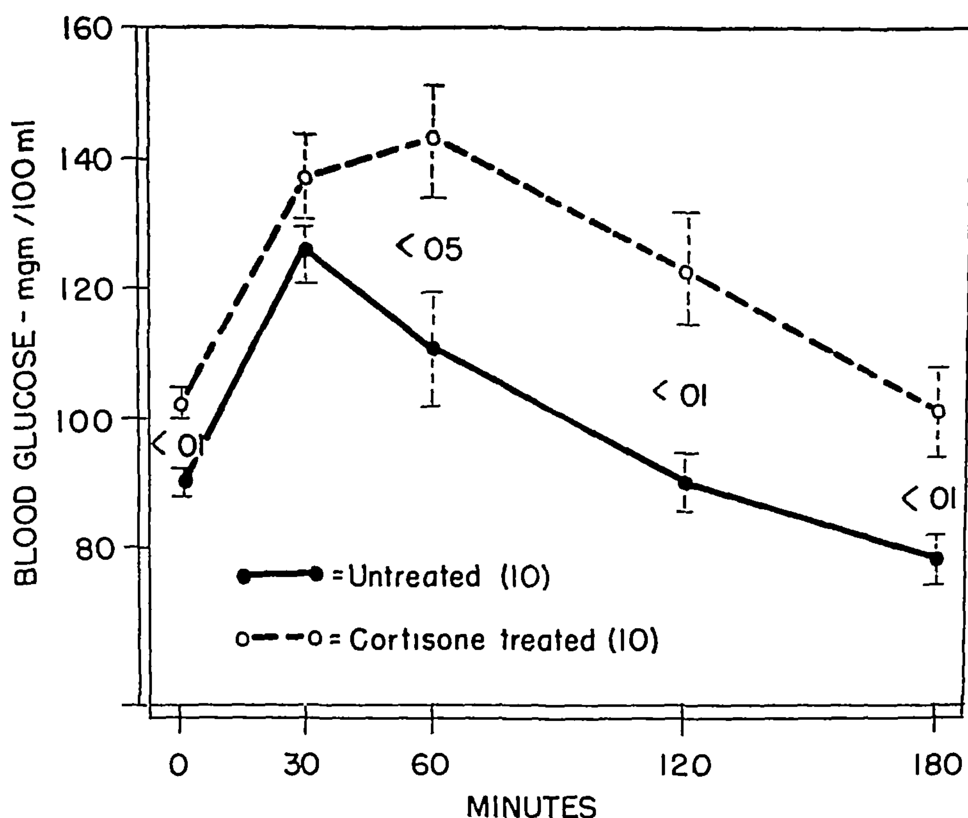


FIGURE 7 *Demonstration that a single oral dose of 200 mg of cortisone acetate 4 hours before an oral glucose tolerance test causes a significant increase in blood sugar at all points of the curve except at 30 minutes In this and subsequent figures bars indicate ± one standard error of the mean*

Figure 7 represents the normal glucose tolerance test in these students and the glucose tolerance of the same students when given 200 mg of cortisone by mouth four hours before the test. You will note that there is a slight but significant impairment of glucose tolerance after cortisone.

Haist. Would it be possible to get a similar effect in normal individuals with previous restriction of diet?

Engel. We put a group of students on a 1400-calorie diet with 150 grams of carbohydrate, which was what we estimated the average anorectic patient in the ward might take, for one week before they had a tolerance test Half the students had a glucose tolerance test at the end of a week, were put on the diet for another week, and then the test was repeated with cortisone. The other half were done the other way around. they got the cortisone the first week, and after another week had a control glucose tolerance test The subjects on the restricted diet showed slightly greater impairment of glucose tolerance, both with and without cortisone, but nothing comparable to that of the ill subjects with cortisone (Figure 10)

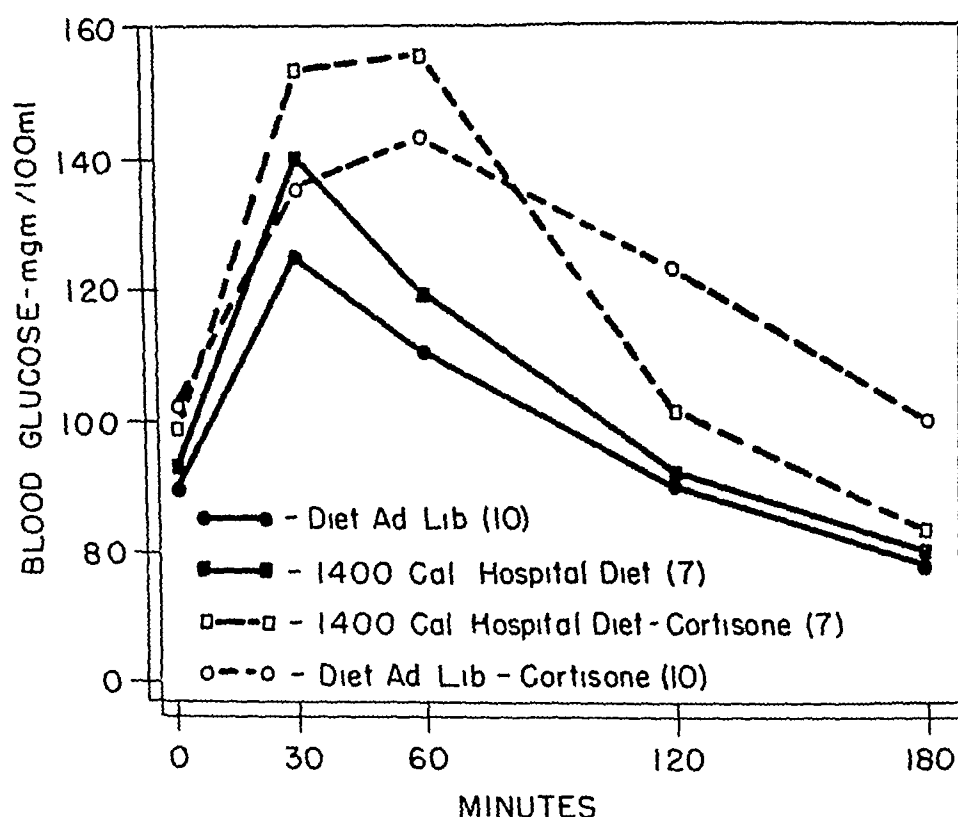


FIGURE 10 Demonstration that limitation of diet to 1400 calories and 150 grams of carbohydrate per day does not significantly modify the response to cortisone in normal subjects

The same result may be seen with a conventional insulin tolerance test, injecting one-tenth of a unit of insulin per kilo intravenously and measuring blood sugar levels at 30-minute intervals

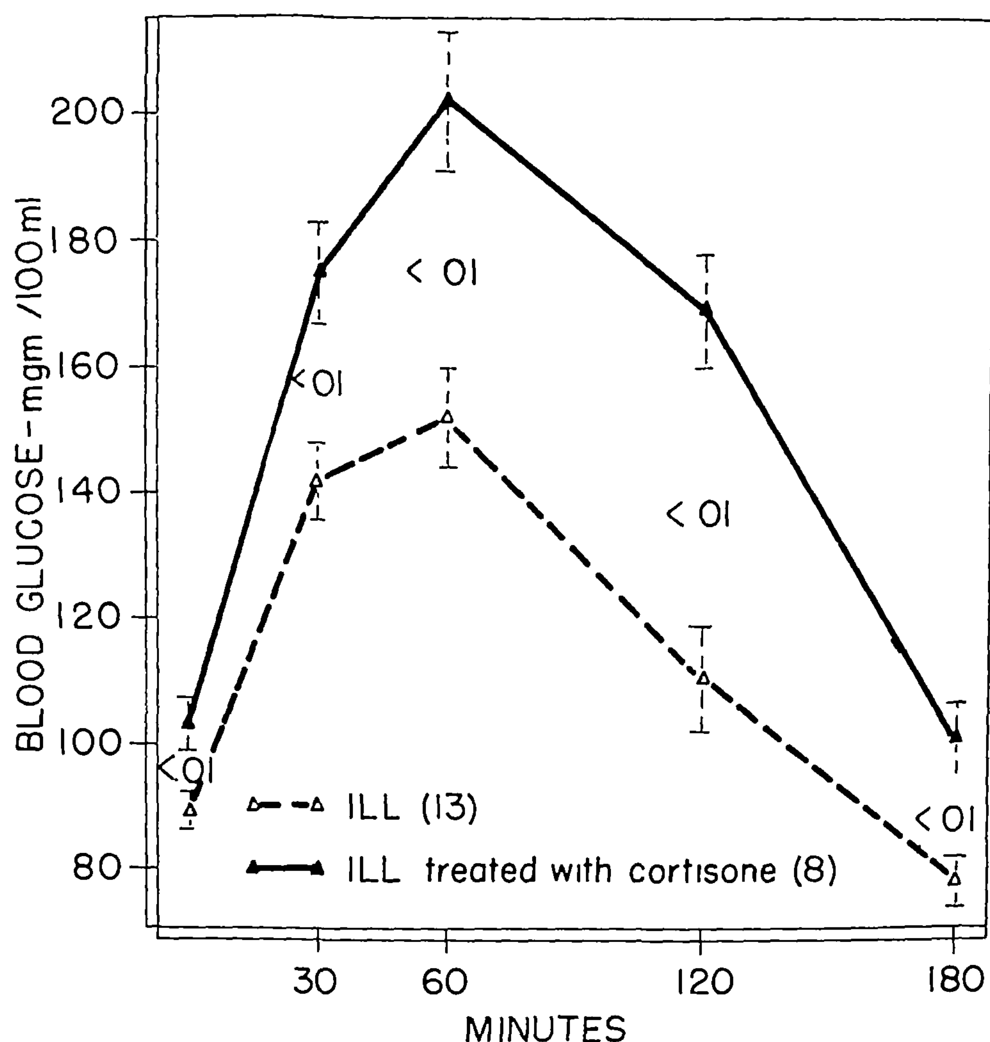


FIGURE 9 Marked impairment of glucose tolerance after single oral dose of cortisone in ill subjects The response to cortisone in this group is significantly greater than in the normal subjects (Figure 7)

tolerance Thus, this does not seem to be an accumulative response. The same thing is true with the insulin tolerance test

von Euler· In general, did these patients have fever?

Engel Some had fever and some did not We were interested in choosing people who were definitely ill and who, we could anticipate, would have abnormal carbohydiate tolerances

von Euler: Most of them did have infectious diseases?

Engel. No There was a fairly bioad spectrum It didn't seem to make a great deal of difference whether the patient had an infectious disease, cardiac disease, disseminated lupus erythematosus, Boeck's sarcoid, or peptic ulcer They responded quite consistently

Burch. Do you think a 1400-calorie diet for a medical student who is ambulatory might be deficient, whereas for a patient in bed in a hospital it might be sufficient?

Engel I don't think so. But I think you have put your finger on one important variable in our study which we have not yet controlled We have been wanting to check it, but it has not been practical to do so Whether the difference in carbohydrate tolerance after cortisone in the two groups could be attributed to their difference in activity, I don't know. I doubt it but I can't be sure

Shorr. We have studied a few normal individuals on prolonged immobilization The reduction in their basal metabolic rate was minimal, on the average 6 9 per cent The discrepancy between activity of the student and that of the ill patient might very well be bridged by the presence of infection and the usual accompanying increase in over-all metabolic activity We found that in order to establish the differences between immobile patients and those who were merely at bed rest, it was necessary to immobilize the subject in a cast up to the chest cage I think there is enough activity during bed rest so that muscle blood flow is not depressed to such a degree as to represent the differences shown

Burton Did you follow the weight changes? Wouldn't they be a good index?

Engel We did not follow the weights of these students

Burton A normal medical student on 1500 calories a day is bound to lose weight It certainly is not adequate, is it, for his calorimetric requirements?

Engel I suspect it depends on the medical student Some are more energetic than others

Green Did you make any serial studies to see whether you had reached the point of maximum response of the student to the diet?

Engel No, we did only one study with the diet We did vary the cortisone dose but not the diet

von Euler What was the dose of insulin given in this study?

Engel One-tenth unit per kilo We have a large number of studies in which we gave various doses, ranging down to one-eighteenth unit per kilo, in a futile attempt to demonstrate that the normal individual has some resistance to insulin after getting cortisone for a day or even for a week From the literature, one gets the impression that resistance to insulin is a quite regular response to cortisone On the other hand, as soon as we did this on the ill people, we found that very quickly, in four hours, they were resistant to insulin

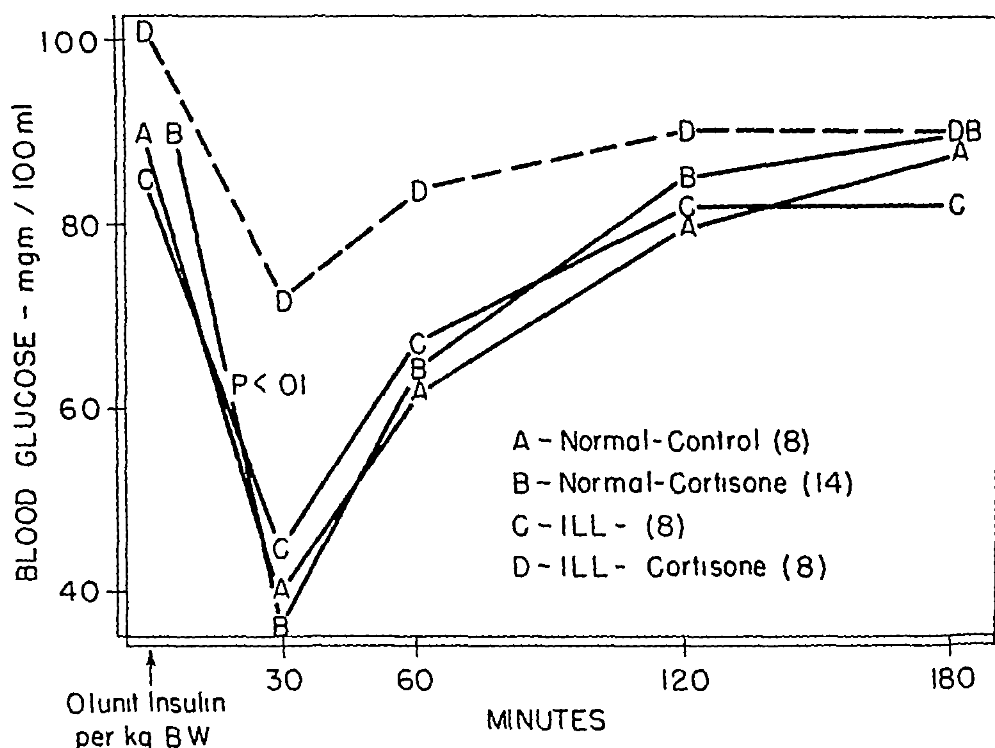


FIGURE 11 Impaired insulin tolerance in cortisone-treated ill subjects compared to lack of effect of either cortisone or illness per se on insulin tolerance

for 180 minutes (Figure 11). In the normal students, 200 mg of cortisone had no effect on the response to insulin. Ill subjects in this study likewise showed an essentially normal response to insulin, but when pretreated with a single dose of cortisone, they exhibited clear-cut insulin insensitivity, the blood sugar falling only to 72 mg. per cent

These data indicate to us that the adrenal cortex intervenes in metabolic responses to injury in a peculiar manner It cannot be said to be the primary factor in producing the alterations in carbohydrate and protein metabolism Rather, there seems to be something intrinsic in the response to injury itself which is potentiated by the presence of adrenal hormone. The individual who is undergoing this reaction to injury, when given cortisone or adrenal hormone in excess of his need, gets a much bigger reaction from it, apparently, than does the normal individual This corresponds fairly well with clinical experience, because it is true that the very ill people who are given ACTH, or cortisone, are usually the ones who are very much more likely to exhibit overdose effects than the relatively healthy people who may take the same dose for a long time.

with respect to ketosis, we have found that the adrenal cortex not only does not stimulate ketosis but actually inhibits it, so that we cannot attribute to adrenal cortical stimulation the change in ketone body metabolism that occurs in response to injury.

Shorr These findings may have a bearing on two aspects of shock People may be exposed to shock either quite suddenly when in an otherwise healthy state, or after a series of stresses such as occur on the battlefront Do these changes represent unfavorable or favorable climates for the recovery from shock, and how do they influence the compensatory responses to a reduction in blood volume from whatever cause? Have you any evidence as to what preliminary conditioning does to an animal exposed to blood loss or to trauma?

Engel We do not have any direct evidence, what we have is rather indirect or negative We certainly know that the adrenalectomized animal who does not get this response behaves poorly, and the depleted organism that doesn't get this response tends to behave less well than the normal healthy organism Certainly the well-nourished healthy young man, for example, who appears to get the metabolic response to injury maximally, seems to do better than do the people who are poorly nourished and who do not get the response But exactly what this response really means in terms of protecting the organism, I have no good idea

Shorr In an animal that has been buffeted about and poorly fed, the same type of shock is produced by exposure to smaller blood loss Whether this results from the progressive exhaustion of the adrenal's capacity to respond is something that is still a matter of controversy A point I should like to bring up, which supports your concept of general, nonhormonally-conditioned, adaptive responses, is that it is possible, by careful training, to get an animal that has been adrenalectomized and maintained on salt without adrenal extracts to withstand a degree of shock which is usually fatal for a normal animal That is correct, is it not, Dr Baez?

Baez Yes

Shorr Will you describe how you carried out this slow conditioning with sublethal drummings?

Baez In adrenalectomized, one per cent salt-maintained rats, mortality reached 80 to 85 per cent at 500 rotations In order to make these animals resistant, they were initially given 100 rotations and the amount of trauma was increased 50 rotations each time until, after four to five weeks, sixty per cent of the rats had

Green Were there any changes in the circulatory responses of the ill and normal groups?

Engel: Not that we could ascertain If anything, the students had more severe reactions to the insulin But I think this might be because the students anticipated a reaction, even though we did not tell them what they were getting We had student controls who were given saline intravenously, and many of them were sure they were having insulin reactions

Haist Would you expect to get the same result from ACTH administration?

Engel Yes We have done a certain number of experiments with ACTH, and pretty much the same response occurs. We have much fewer data on ACTH, though, than we do on cortisone.

Haist There seem to be some differences in the stimulating effects of ACTH and cortisone on the islands of Langerhans.

Engel I wonder whether that is due to the fact that just about every preparation of ACTH is fairly well contaminated with growth hormone

Haist. That I couldn't say

FACTORS INFLUENCING THE RESPONSE TO SHOCK-INDUCING AGENTS

Engel One might say that all this is a long cry from shock, but I think it does have a bearing on certain questions For example, does the adrenal have something to do with the body's ability to respond to an agent which might induce shock, and does giving adrenal hormone to prevent shock do any good? As regards the latter question, I should be rather inclined to say from the data I've discussed that it is probably best not to meddle, that these reactions are proceeding by some determinant other than the adrenal cortex, and that by adding adrenal hormone beyond that which the body is already supplying, homeostasis might actually be upset rather than preserved

I also wish to point out that from the various things we have studied we have reason to believe that not all the metabolic actions I have mentioned were permissive reactions of the adrenal cortex One example is the ketosis and fatty liver that is associated with injury In the case of fatty liver, Levin (8) and others have raised serious questions about whether the adrenal is concerned with this in a primary sense Actually, some factor from the anterior pituitary seems involved Possibly growth hormone is the important factor, but the adrenal hormone has to be present for it to happen Also,

of this period, and for several days thereafter, these rats are resistant to as many as 1500 drummings, a 100 per cent lethal exposure for normal untrained rats. Animals which are made progressively resistant over a period of eight to ten days maintain their resistance to drum trauma for as long as two to three weeks.

Stead. I should like to know what a rat does while he is being drummed.

Zweifach. The animals are placed in the drum with their paws taped together so that they cannot grasp the walls of the drum. As the drum rotates, the rat is carried along on a wedge until, by gravity, he falls and strikes against the surface of the opposite side of the drum. On the average, the rat falls about 10 inches. This type of tumbling and falling is repeated at the rate of about 45 per minute. After several rotations, the falls appear to be uniform, with the rat apparently in a dazed, limp state.

Moe. If this were a learning process, it would hardly explain the cross tolerance to other varieties of shocking stimuli.

Nickerson. Has an attempt been made to condition animals by repeated sublethal hemorrhages over a period of time? If this procedure produces resistance to subsequent trauma, it would answer a number of questions which have been raised.

Shorr. It has often come to our minds but has never been explored. It should be.

Green. The question was raised a minute ago as to whether repeated bleedings increase the tolerance to bleeding. I do not know of any work that has been done over a period of days or weeks, but there was a study during the last war in which successive bleedings were done during approximately a day, followed in each case by reinfusion. In each successive bleeding, the dogs had a smaller bleeding volume. In other words, the bleedings decreased rather than increased the tolerance (9).

Fremont-Smith. Has conditioning by means of straight exercise been tried?

Zweifach. Several regimens of muscular exercise have been explored without inducing the phenomenon of resistance. Likewise unsuccessful was simple rotation without trauma. Padding the muscular parts of the body serves to prevent the development of resistance. Apparently, a definite degree of muscle trauma must be involved in this type of conditioning.

Baez. When normal rats are provided with a protective padding of the abdominal wall, so as to prevent direct visceral exposure to trauma, and are subjected to increasing numbers of rotations in

developed resistance to 700 to 750 rotations of the drum The remaining 40 per cent died after 600 or 700 rotations

Shorr: In other words, resistance was achieved by smaller increases in the number of drummings to which they were progressively exposed So the capacity for resistance is inherent in certain nonadrenal, hormonally-conditioned reactions of the body.

Engel Do you think the phenomenon of resistance, as studied by this technique, is the same as that by which the animal protects itself from its first injury?

Zweifach Such a distinction is important The mechanisms by which the rat progressively becomes resistant to the lethal effects of drum trauma may be basically different from the adaptive reflexes which enable the animal to combat successfully the initial traumatic insult

Moe How long did it take to condition these adrenalectomized, salt-maintained rats?

Baez It took twice as long to condition the adrenalectomized rats to tolerate 700 revolutions in the drum as it took for the normal rats, since we increased the rotations by 50 each time with the adrenalectomized rats and by 100 with the controls

Moe Would they go into failure after you were through with them?

Zweifach The rats used in these experiments were maintained after adrenalectomy by supplementing their diet with one per cent sodium chloride in the drinking water Such animals survived for six or more months in apparently good health Trauma resistant, adrenalectomized rats will die within two to three days when deprived of supplementary salt, just as do control, undrummed, adrenalectomized rats The development of resistance to trauma is not accompanied by resistance to the effects of adrenal insufficiency per se

Nickerson. An animal might develop, over the period of training, an ability to adjust his metabolism and become a little more resistant However, can we rule out a more mechanical conditioning, something like the formation of callus on the hands? Could there be simply a strengthening of the connective tissue in fascial planes so that a revolution of the drum no longer gives the same kind of jolt to an internal organ as before?

Zweifach I doubt whether this is the type of factor which is operating Such an explanation would not account for the development of resistance to drum trauma by repeated sublethal drummings over a period as short as seven to eight hours At the end

of this period, and for several days thereafter, these rats are resistant to as many as 1500 drummings, a 100 per cent lethal exposure for normal untrained rats. Animals which are made progressively resistant over a period of eight to ten days maintain their resistance to drum trauma for as long as two to three weeks

Stead I should like to know what a rat does while he is being drummed

Zweifach The animals are placed in the drum with their paws taped together so that they cannot grasp the walls of the drum As the drum rotates, the rat is carried along on a wedge until, by gravity, he falls and strikes against the surface of the opposite side of the drum On the average, the rat falls about 10 inches This type of tumbling and falling is repeated at the rate of about 45 per minute After several rotations, the falls appear to be uniform, with the rat apparently in a dazed, limp state

Moe. If this were a learning process, it would hardly explain the cross tolerance to other varieties of shocking stimuli

Nickerson Has an attempt been made to condition animals by repeated sublethal hemorrhages over a period of time? If this procedure produces resistance to subsequent trauma, it would answer a number of questions which have been raised

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Baez When normal rats are provided with a protective padding of the abdominal wall, so as to prevent direct visceral exposure to trauma, and are subjected to increasing numbers of rotations in

the drum, these animals also develop resistance We exposed the rats in the drum every other day, increasing the number of rotations by 100 each time Forty-eight hours after the last drumming of 1000 rotations with padding, they were exposed to 1000 and 1200 rotations without the abdominal protection, and were found to withstand this trauma

Shorr: In other words, padding in a certain manner permits the development of resistance

Baez Yes We tried to protect the abdominal viscera as well as we could, padding the abdomen with foam rubber about ¼ inch thick When rotated in the drum or exposed to standard hemorrhagic procedures, we found they had developed resistance to these types of stress

Fremont-Smith Do I gather, then, that if padded completely, they do not develop resistance, but if the abdomen only is padded, and the other muscles exposed to trauma, they do develop a resistance? Is that the distinction?

Baez: We have no experience with complete padding of the animal I referred to padding of the abdomen only, leaving the rest of the muscles exposed to trauma

Shorr Wasn't that done under anesthesia?

Baez I think they were anesthetized animals

Shorr That might very likely be the determining factor

INTERMEDIARY METABOLISM DURING SHOCK

Engel We might now go on to consider some of the changes which are more characteristic of the stages about which there is agreement that shock has developed Figure 12 is a "road map" of intermediary metabolism in shock What I tried to do in this diagram was to indicate both concentrations or amounts of certain metabolites in tissue or body fluid as well as hypothetical rates of reaction in either direction, the amounts being indicated by the size of the bars, and the rates of the reaction by the size of the arrows, irrespective of direction It will be seen that all these arrows are two-way. The three different arrows at each point represent the normal state and early and late shock It should be emphasized that these comparisons are relative and quite arbitrary, they do not represent precise quantitative relationships Also, I have divided the diagram into certain arbitrary compartments in which some of the main metabolic changes take place, i e blood, muscle, and liver This was not done with the intention of ignoring other organs, but these, in our experience at least, turn

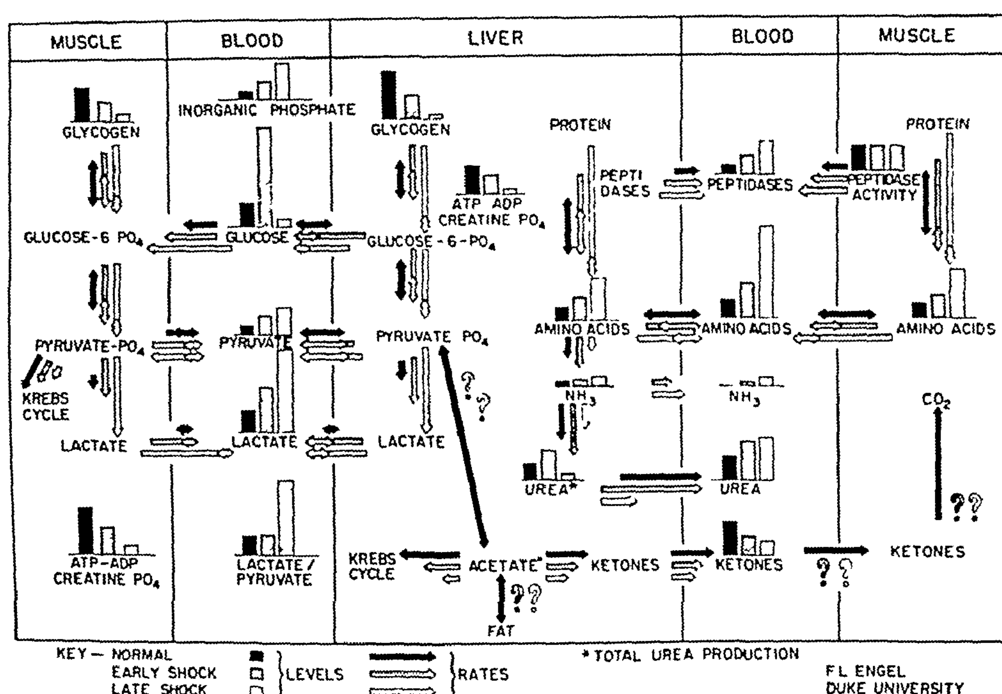


FIGURE 12 Intermediary metabolism during shock Reprinted, by permission, from Engel, F L The significance of the metabolic changes during shock *Ann New York Acad Sc* **55**, *381 (1952)*

out to be the organ systems which make the greatest contribution to the changes measurable in the blood

I shall begin by describing some of the changes in the blood that one sees when an animal is subjected to shock, and then go on from that to consider the evidence for contributions by different organs, organ systems, or tissues to the changes in the blood To consider carbohydrate metabolism first the earliest change, as already mentioned, is a rise in blood sugar resulting from liver glycogenolysis It may rise to extremely high levels, and the level which it reaches in any species depends to a considerable degree on how much liver glycogen there is The rat which has been fasted and then put on an ordinary chow diet, which is not very high in protein, will have a modest rise in blood sugar in response to certain types of injury If that same rat is put on a high protein diet for some time and then is fasted, it will have a much greater rise in blood sugar after injury

The dog, which is accustomed to a high protein diet, may have extremely rapid rises in blood sugar, up to 200 mg per 100 gm or more Obviously, if the animal or individual has recently eaten and has a high liver glycogen, the blood sugar will rise to much higher levels

Zweifach: In subdividing the shock syndrome into early and late, are you using an arbitrary method of determining the onset of these phases, or does the latter phase represent the hypotensive state?

Engel. That is a bad thing to try to be specific about at this Conference

Zweifach I think it would be helpful to define certain basic terms with reference to the many metabolic changes which are being presented Are these metabolic derangements related to shock, to hypotension per se, or only to the specific experimental procedure being used?

Engel I think the best that can be said is that the changes classified as late shock are in general the things that are happening after the animal has been hypotensive for a considerable period of time and it can be anticipated that he is going to die before long Late shock may be considered as synonymous with irreversible shock

Zweifach: Will some of these metabolic changes appear, for example, in a man who has been subjected to phlebotomy, without any change in blood pressure?

Engel. None of the late changes will, some of the early ones will

Zweifach: In other words, hypotension is not a necessary prerequisite for many of these metabolic alterations

Engel For the blood sugar change, I would say no, for some of the others, I suspect it is When we deal with the changes in early shock, we are on the borderline of that no-man's-land I referred to before, where it is very difficult to classify either the metabolic changes or the circulatory changes in terms of their precise meaning

Shorr Most of these data are on hemorrhagic shock?

Engel: Yes

Shorr: The late stage is that in which the animal is entering the phase of circulatory deterioration?

Engel Yes

Fine. Is shock induced abruptly or slowly?

Engel This, of course, also influences the result If the shock is induced abruptly, many of these things occur much sooner. When I say shock, I mean a situation in which circulatory deterioration occurs rapidly, induced say, by hemorrhage in 30 to 60 minutes instead of over five hours In the former, the changes may or may not develop early There may be quite a lag before they take place, and then they develop more rapidly, or they may begin almost

immediately with the bleeding. On the other hand, if the same hemorrhage is extended over four or five hours, the early changes may only begin to occur after four to five hours and the late changes some hours afterwards This is all in the way of semantics as to how the experiment is defined

Fine. It is not altogether semantics, for Dr. Long (10) described the depletion of cholesterol and ascorbic acid in a rat which was bled two per cent of its body weight over a period of four hours My question is whether that depletion would occur if the adrenals were abruptly put out of circulation, so to speak, by rapid bleeding, to the extent of four per cent of the body weight, which is the way we produce hemorrhagic shock I have reason to question whether one could deplete the adrenal of its cholesterol and ascorbic acid by such rapid bleeding, because when we applied Seligman's stain for ketosteroids in the adrenal cortex, we did not see a depletion of ketosteroid in abrupt bleeding, but we did see it in such stresses as starvation, irradiation with x-ray, and exposure to cold. Therefore, the speed of induction of shock may affect the response of a variety of metabolic processes

Engel It certainly affects the result temporarily, but I think it is fair to say that, with certain exceptions which I will mention in the course of the description of these changes, in our experience just about everything that we have seen in the normal animal well along in shock occurs in the adrenalectomized animal, too Relatively few of the changes given in Figure 12 are dependent on the adrenal The only one that I think of is the initial rise in blood sugar In the absence of the adrenal medulla or the adrenal cortex, this is not found, the blood sugar falling progressively All the other changes occur, although it is true that quantitatively they might not occur to exactly the same degree that they occur in a normal animal, nonetheless, they do occur

Your point is very well taken, certainly as far as those reactions which are dependent to some degree upon the presence of an intact adrenal cortex are concerned

Burch Is there any difference, as far as you know, between the so-called late shock and the state of any dying animal regardless of the etiology of the dying state?

Engel I would strongly suspect not, because I think that almost everybody dies in shock eventually

Burch Is your definition of late shock the state of any patient or any animal that is dying?

Engel It could be

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death under all conditions I mention it only because we have found that we could more or less draw a line to separate so-called late shock from early shock by the change in amino acids If no change in amino acids occurred, we couldn't be sure that the animal was getting to the later stage of shock, whereas if there were a rise in amino acids, we had reasonable confidence that this was so

Fine. Do you interpret the rise in amino acids as a metabolic phenomenon, or may it possibly result from slow flow in the peripheral circulation?

Engel: I think it is a combination of both speed of circulation and metabolic changes in muscle and in liver One of our problems in this discussion will be to attempt to differentiate between the effects of phenomena which are purely secondary to changes in rate of circulation and those which are due to actual changes in metabolism in the organ or tissue I hope to get into that in a minute

One of the changes that has interested us recently has been studied in my laboratory by Dr T B Schwartz the change in proteolytic enzymatic activity of plasma in rats during hemorrhage and shock We were curious about this from the standpoint of whether there might be correlation between the rise in plasma amino nitrogen and enzymatic activity, since there is evidence that the latter reflects, in part, an enhanced role of protein catabolism in muscle and elsewhere Other investigators have measured peptidase activity in serum, but all such studies dealt either with muscle trauma, as in the case of Green and Stoner's work (11), or with burns, as in the case of Zamecnik's work (12) In both cases, the significance of the observed changes was obscured by the problem of direct release of enzymes from the mechanically damaged tissue, and by the fact that in both these types of experiments there was a considerable element of hemolysis Since the red cell contains about twenty times as much peptidase activity as the plasma (13), it is difficult to interpret changes in plasma peptidase activity in the presence of hemolysis

The results of Dr Schwartz's study on the peptidase activity in nonhemolyzed plasma in hemorrhagic shock are shown in Figure 13 It demonstrates that peptidase activity does rise under circumstances where we are not dealing with direct muscle trauma or hemolysis We have not done too much on the source of the enzyme, but I will show some data later on to indicate the possibility that it may come, in part at least, from muscle

Burch Are we concerned with the study of the chemistry of impending death?

Engel If we are measuring impending death, then the late changes are not very important perhaps. I think that nobody has established whether these changes that we study in shock really precede impending death whether the individual dies because of these changes or whether these changes are taking place because he is beginning to die

Zweifach What is meant by impending death? Is the state of shock a continuous spectrum or are there discrete compartments that can be ruled off? Can it be said that when a patient is dying, one set of metabolic derangements is put into motion, in contrast to the progressive array of changes which develop during stress and become exaggerated as the condition persists?

Engel I suspect it is probably the latter. Maybe we can come to more agreement about that after discussing the changes that take place

In addition to the rise in blood sugar, which is most striking during the early phases and lasts well into shock, a fall in blood sugar eventually occurs in the normal animal This is decidedly a very late change, except in the adrenalectomized or demedullated animal in which it begins very early There are changes in blood pyruvate and lactate, with comparable rises in both, until the late stage of shock when the blood sugar is falling and the lactate to pyruvate ratio increasing because of a more rapid rise in lactate. The degree to which these occur is again very much determined by how the shock is induced, i e , hemorrhage, muscle clamps, or tourniquet Some of these changes are much more striking in tourniquet shock, or with other techniques involving muscle damage, than in hemorrhagic shock The increase in the lactate-pyruvate ratio has been interpreted as suggestive of a shift to anaerobic carbohydrate metabolism

In protein metabolism, the rise in blood amino acid nitrogen occurs fairly early and is progressive in the rat if irreversible shock is induced In other species, it is much less prominent, although in most some change in amino acids is eventually found if the process is followed long enough In the rat in hemorrhagic shock, it has been a sensitive indicator of the course of shock I have never seen a rat having a significant elevation in blood amino nitrogen that survived without treatment. With treatment, this, of course, is different, and I do not mean to imply that a rise in amino acid is a cause of death or that it is necessarily absolutely indicative of

and the circulation We recently carried out studies on blood ketone bodies in rats during hemorrhagic shock In the course of these studies, we found that fasting ketonemia is extraordinarily sensitive to changes in the circulation.

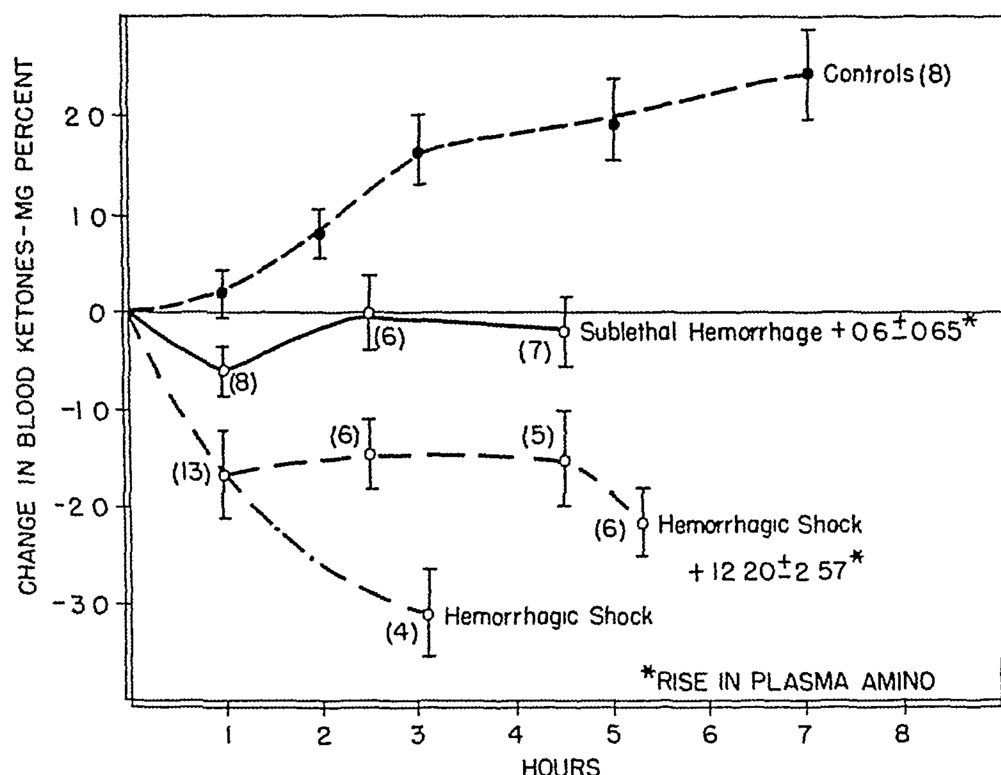


FIGURE 14 Change in blood ketone levels during sublethal hemorrhage and shock compared with that in rats fasted a comparable period

The animals in the experiments of Figure 14 were all fasted 24 hours, and then experimental measurements were begun. As can be seen, the normal animal in the course of a further seven hours of fasting showed a gradual rise in blood ketones, as one would expect Even a small hemorrhage, one which was associated with no rise in plasma amino acids and following which the animals did not appear particularly ill, was associated with a suppression of fasting ketosis Parenthetically, I think this phenomenon has a bearing on the results of many studies on ketosis in the rat. Anything that interferes with the circulation or produces a slight respiratory hypoxia may quickly suppress ketosis. This is particularly true in adrenalectomized rats, and may account for the common finding that ketosis does not readily develop in the untreated adrenalectomized animal In animals that were bled into shock, with a mean rise of plasma amino nitrogen of 12 20 mg per 100

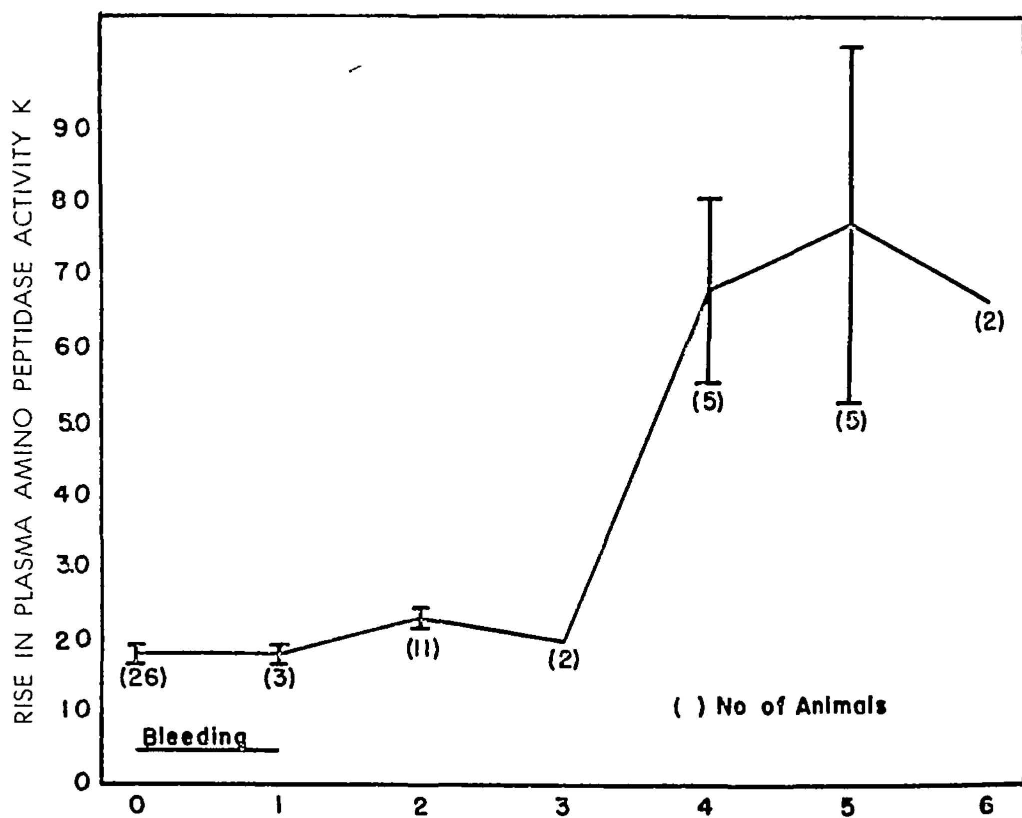


FIGURE 13 Rat plasma glycylglycylglycine activity in hemorrhagic shock Plasma amino peptidase expressed as millimols of glycylglycylglycine split per hour by 0 1 ml plasma per one ml of hydrolysis mixture (0 5 ml of 0 1 mM GGG, 0 5 ml of 0 002 mM barbital buffer pH 7 8 and 0 5 ml of 0 85% NaCl) during hemorrhagic shock Data expressed as mean ± S E Rats were bled 3% of body weight during the first hour and 0 5 to 1 0 ml. every one to two hours thereafter until death

In addition to changes in blood amino nitrogen, small changes in blood ammonia levels in hemorrhagic shock have been reported by various investigators, but they have never been striking. I believe the maximum rise that Wilhelmi, *et al* (14) found was something of the order of a half milligram per 100 gm in the plasma, and they found that, in general, the concentration of ammonia in plasma was lower than tissue ammonia levels prior to shock It is unlikely therefore, that blood ammonia elevations are of any significance from the standpoint of toxic effects

The changes in blood urea are complicated by the fact that we are dealing with both renal and hepatic phenomena here. The blood urea goes up steadily in shock because of renal failure, but the rates of urea production by the liver vary, being increased at first and then decreased as hepatic circulation fails

Finally, we come to fat metabolism There has been practically nothing published on changes in fat metabolism in relation to shock

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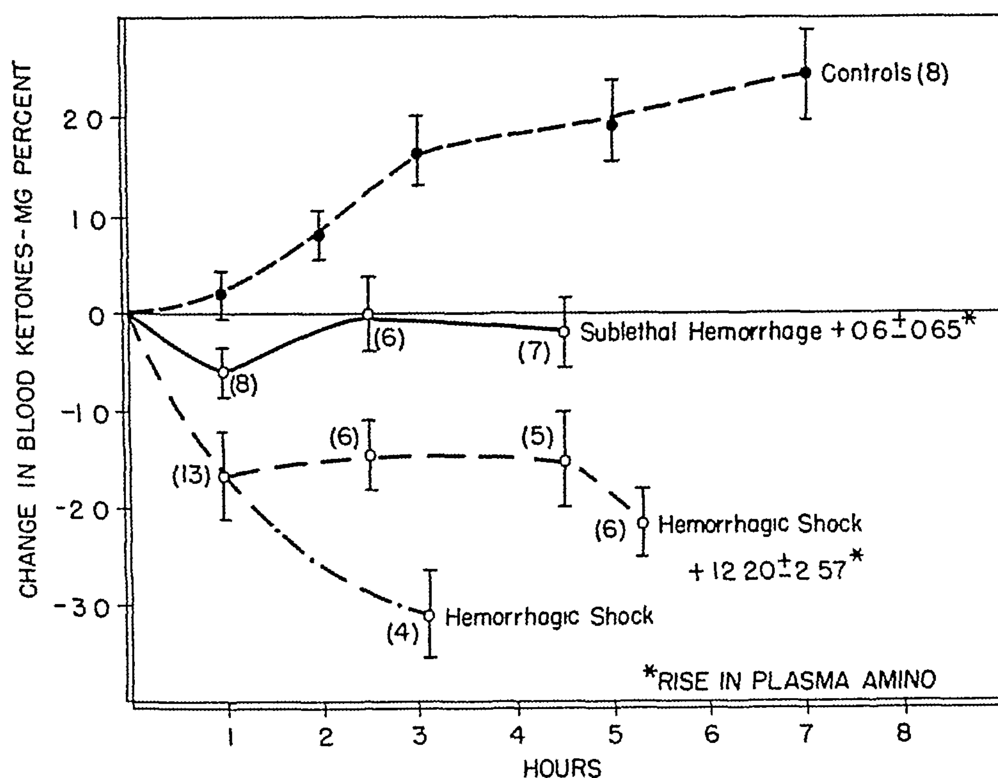


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gm , ketone levels go down quite sharply This same phenomenon occurs in adrenalectomized animals, so it is not due to stimulation of the adrenal cortex, and also probably it is not due to epinephrine We have found that in the normal rat an injection of epinephrine does cause a decrease in ketone body levels at the first hour, but then at two or three hours the ketones are definitely increased Interestingly enough, when epinephrine is given to adrenalectomized animals, it causes a continual decrease in the ketone levels This is not a dose of epinephrine sufficient to kill adrenalectomized animals

There are some peculiar things about the circulation and ketone body metabolism which deserve investigation. For example, Janes (15) has reported that nicotinic acid administered intraperitoneally causes a marked rise in blood ketones, while nicotinamide does not More recently, he found that Priscoline will do the same thing This makes me wonder just what the circulation through the liver has to do with ketone body formation

Stead I thought that people with Addison's disease didn't get ketosis easily Am I right or wrong?

Engel I don't think that is right That impression may be related to the fact that the circulation is bad, and this, rather than adrenal insufficiency, may suppress ketosis I have seen one patient with diabetes and Addison's disease come into the hospital in marked hypoglycemia and with very high levels of blood ketones I am not sure that there can't be ketosis in the absence of the adrenal

Burch The general mechanism of this is not clear to me. Do you interpret this to indicate a decrease in the rate of destruction of ketone bodies or an increase in the rate of production?

Engel Up to this point, we haven't interpreted it The effect of infusion of sodium octanoate on ketone levels before and after shock does not answer this point conclusively but strongly suggests that ketone body production by the liver may be the important point of influence by shock

Green. If I followed you correctly, you said that a single injection of epinephrine would lower the ketones and that afterwards they would go up

Engel: Yes

Green If you gave a continuous infusion of epinephrine, would they go down and stay down?

Engel We haven't done that

Cotzias. How do you measure amino nitrogen in the blood?

Engel We have used the colorimetric method of Frame, Russell, and Wilhelmi (16) Studies done with the Van Slyke method have indicated rises, but there has not always been a one to one correlation with the colorimetric method.

Cotzias That is very interesting because it is quite possible that a small part of the amino nitrogen might be in the form of amines, which might, of course, explain very many of the pharmacologic findings

Engel I think Dr Haist had some data on the comparison of the Van Slyke and the Frame method.

Haist Yes, there was a lesser rise in amino nitrogen when determined by the ninhydrin procedure than by the modified method of Danielson (17)

Engel And I should say that in his studies on proteolytic enzyme activity, Dr Schwartz measured plasma amino nitrogen by the colorimetric ninhydrin method, the modification of Moore and Stein There were good rises by this method, which measures amino acids more specifically

Cotzias No, it doesn't. I don't think it is much more specific than the Frame method

Engel Oh, you don't?

Cotzias With the Stein and Moore method, even ammonia will give just about the same color as amino acids

Engel Yes of course Ammonia, I should have mentioned, is an important exception there We do know, however, that the concentration of ammonia in the blood even in late shock is so small that it could not contribute much

Cotzias I wonder whether tyramine or tryptamine might contribute to some of the phenomena that Drs Shorr and Zweifach have been studying during the last few years

Engel I think it is very important to use methods that would measure other amines

SIGNIFICANCE OF THE METABOLIC CHANGES IN SHOCK

Shorr To what degree do you think these changes represent critical or specific episodes in the shock syndrome? Perhaps you might express your own judgment on that score as you describe the phenomena

Engel The changes described thus far are relatively uncontroversial in the sense that everybody has measured these things and seen them Where disagreement does arise is in considering their meaning, particularly their meaning in terms of the metabolic

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Green. If I followed you correctly, you said that a single injection of epinephrine would lower the ketones and that afterwards they would go up.

Engel. Yes.

Green. If you gave a continuous infusion of epinephrine, would they go down and stay down?

Engel. We haven't done that.

Cotzias. How do you measure amino nitrogen in the blood?

venous oxygen saturation and the rise in the blood amino nitrogen The correlation was better than that of the blood amino nitrogen and the peripheral vein oxygen saturation. We also found a fairly good correlation between the fall in blood pressure and the rise in amino nitrogen in the cat, as well as in the rat

When a comparison was made between the behavior of liver slices from rats which had been similarly bled, and the rise in blood amino nitrogen, we found essentially the same correlation, namely, that the higher the rise in blood amino nitrogen, the greater was the depression in the total oxygen consumption of the liver slice These latter data suggest that we are dealing with something more than simply a failure of amino acids to circulate to the liver, by which I do not mean to imply that this something more is necessarily critical

Shorr May I call attention to species differences In our laboratory, the oxygen consumption of the liver slice of the dog was followed throughout the compensatory and decompensatory phases of shock into irreversibility, and over-all depression in oxygen consumption was only about 15 per cent There can be a profound deterioration of the circulatory responses of the animal with a retention of the oxidative mechanisms

Engel This is true, also, of the cat, for which Wilhelmi and Russell made measurements It is interesting that they found this failure to exhibit a fall in Q_{O_2} true in a cat that was fasted overnight, but if they fasted the cat for 48 hours, the response was just like that in the rat This brings up two points The first is that the behavior is going to depend on certain intrinsic metabolic characteristics of the species, as well as on previous diet and other factors The second is that no one should jump to the conclusion, from data such as these, that the liver has failed with respect to its over-all oxidative capacity, and that this is responsible for what happens in shock

When certain other parameters of amino acid metabolism were measured, again both in the intact animal and in slices, further significant correlations were found We tackled the problem of urea formation by a technique essentially similar to the one which was used in the studies of the adrenal cortex which I spoke of earlier, namely, studies on rate of accumulation of urea nitrogen in the blood, in this case after infusions of amino acid solutions (20) A continuous infusion of an amino acid mixture over three hours at the rate of one millimeter per hour was used The animals were nephrectomized the night before, and a control rate of urea forma-

integrity of different organs Two basic considerations must be kept in mind, even though our experimental data do not always enable us to differentiate between them When there are metabolic changes which we wish to attribute to something which happens to an organ such as the liver, are they due to the fact that the blood doesn't flow through the liver fast enough, and, therefore, there is a sort of prehepatic failure (or pre-renal failure, or pre-any-other-organ-failure), or do these changes occur because intrinsic abnormalities in the organ have developed which interfere with the metabolism of substances that do circulate through the organ? I think Dr Fine put his finger on this a little earlier, when he said that it can be extraordinarily difficult to differentiate these points.

In the work done at Yale, we tried to get at some of these points by doing simultaneous studies, in the case of the liver, for example, by several different techniques One involved the study of certain metabolites in the blood, and another the study of comparable changes in those metabolites when various procedures were done to impair the circulation through the liver And, finally, comparable studies were done on the metabolism of the liver slice *in vitro*, both after the circulation had been restricted by shock and after mechanical blocking of the circulation to the liver I do think that the combination of those approaches leads to safer ground in the interpretation of certain metabolic changes than if only one of those things had been studied

The area concerning which we have the most information is nitrogen balance. A striking change that occurred in our experiments was a rise in plasma amino nitrogen It is known that the plasma amino nitrogen has its origin in all tissues but that the organ which is most concerned with regulating its level is the liver The liver assimilates amino acids, either by deamination with consequent urea formation, or by incorporation into the cell as free amino acids, as peptides, or protein Liver protein is also continually turning over and may contribute amino acids in its breakdown Other tissues, such as muscle and kidney, are assimilating and releasing amino acids, too, but the liver is the main site of deamination

I shall now present data which suggest that something happening to the circulation of the liver has to do with the changes in plasma and whole blood amino nitrogen during hemorrhage and shock (18,19) The level of the portal venous oxygen saturation was measured in rats bled progressively over several hours into shock, and these levels were compared to those of the blood amino nitrogen A good correlation was found between the fall in the portal

in situ by a procedure which we devised of removing the entire gastrointestinal tract and leaving the circulation through the hepatic artery intact, such a liver has good respiration and good ability to keep amino acids from accumulating in the blood If a clamp is put on the hepatic artery and the liver left totally anoxic for varying degrees of time, the liver will lose its subsequent ability to make urea and to perform other functions, depending on how long it was anoxic Actually, the resistance is surprisingly great Even after two hours of anoxia, the liver has a pretty good ability to recover eventually (18)

Shorr One of the classic experiments of Van Slyke's group showed the manner in which the blood pressure, which is apparently the determinant of flow, influences liver functions If one observes either the plasma ammonia nitrogen or the uric acid level in relation to the degree of hypotension produced, at about 50 mm pressure, a continuity of the normal levels is seen and then with the shift down to 30 mm pressure, a rapid rise in these constituents occurs If, after about one hour, the blood pressure is restored by transfusion, these circumstances are completely altered In the dog, at any rate, the defects occurring at this degree of hypotension for this period of time are corrected once the blood pressure has been restored by transfusion

I think that Dr Fine also has some studies, if I am not mistaken, in animals that went on to circulatory collapse but were able to correct these defects temporarily Do I recall our conversation correctly?

Fine· We found that even after the dog was no longer responsive to transfusion, it could still deaminate individual amino acids injected into the blood stream about as well as before the transfusion The degree of depression of deamination in the period prior to transfusion could not be attributed so much to failure of liver function as to poor blood flow Because it is difficult to determine these matters by inferential data, we moved to the measurement of a specific function of the liver that could not be confused with the functions of other tissues, the capacity of the liver to make fibrinogen and prothrombin Those data verify Dr Engel's view that there is indeed a severe functional impairment of the liver After they have recovered from shock, dogs show a very much delayed capacity to restore a depleted fibrinogen level to a normal concentration The same is true of prothrombin

Shorr So the question is, as I see it, the relationship of each of these defects in terms of their reversibility or irreversibility to the

tion was calculated in control animals, prior to infusion Then, groups of animals were (*a*) not bled, (*b*) bled amounts which resulted in lethal shock, or (*c*) bled a small hemorrhage which did not result in death The total amount of urea nitrogen formed in the subsequent three-hour period was estimated by measuring the increase in blood urea

It was found, first of all, that following the infusion of the amino acids into these animals, the normal animals formed considerable amounts of urea in the subsequent three hours In the animal that had had a small hemorrhage, the amount of urea formed was close to normal at first but then increased over that of the normal animal, indicating that early in the response to hemorrhage, the liver certainly had a normal capacity for making urea. The increased urea formation presumably was from tissue amino acids released into the blood at relatively high rates In animals that had died in shock, on the other hand, the rate of urea nitrogen formation was severely depressed We cannot say whether this was so because the blood was flowing too slowly or whether the liver was in too unhealthy a condition

Confirmation of the depression in urea synthesis is found in the data of Wilhelmi on the *in vitro* synthesis of urea nitrogen from ammonium lactate, with and without ornithine, in liver slices from animals in shock Mild, moderate, and severe degrees of shock were defined in terms of the rise in blood amino nitrogen the moderate were those that had a fair rise, and the severe were the ones that had a marked increase The decrease in urea synthesis *in vitro* was found to correspond closely with the degree of shock as reflected in the blood amino nitrogen levels

Moe This could still be the result of the prior period of anoxia of the liver of that animal, couldn't it?

Engel Yes, it could, and I will present some data on that point shortly The best one can say is that when these tissue slices were put in a medium with oxygen, as they were in our work on the shocked animals, they did not recover their ability to make urea to the same degree they had possessed before There was some hangover from the effect on the liver of, presumably, hypoxia

Moe If the liver slice is exposed to an anaerobic medium for a period of time, and then re-exposed to oxygen, is there a residual depression? Is that what you are implying?

Engel Yes, we have quantitative data which indicate that when a normal liver slice is put in a completely anaerobic medium, the synthesis of urea is depressed Also, if the liver is made anoxic

in situ by a procedure which we devised of removing the entire gastrointestinal tract and leaving the circulation through the hepatic artery intact, such a liver has good respiration and good ability to keep amino acids from accumulating in the blood If a clamp is put on the hepatic artery and the liver left totally anoxic for varying degrees of time, the liver will lose its subsequent ability to make urea and to perform other functions, depending on how long it was anoxic Actually, the resistance is surprisingly great Even after two hours of anoxia, the liver has a pretty good ability to recover eventually (18)

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Shorr So the question is, as I see it, the relationship of each of these defects in terms of their reversibility or irreversibility to the

specific circulatory pattern manifested by animals that are going on to progressive failure If they do not reverse, they may be potentially significant for the progression of the syndrome There are dichotomies of functional activities, and our concern is the specificity of the phenomena that continue to persist after temporary restoration of blood volume and blood pressure, to the sequence of progressive circulatory collapse I would guess that there is forcibly reduced blood flow in the liver in many human diseases, not clear down to the shock level but low enough so that this might be treated as a common factor in various circumstances I would beg that we keep in mind two variable problems the forced reduction in blood flow through the liver, and the interference with the hepatic enzyme systems and whether the enzyme systems recover

Engel I certainly agree it is very important to get more data on how many of these functions are affected and in what direction, when the circulation is restored even temporarily.

Haist With respect to irreversibility in relation to blood pressure, if there is a large mass of anoxic tissue outside the liver, there may be changes in amino nitrogen despite the fact that the systemic blood pressure is maintained by infusion of fluid. I would suggest, therefore, that the persistence of changes may be related to anoxia of extrahepatic tissue

Shorr That is true.

Engel Table I gives data from Wilhelmi, Russell, Engel, and Long (14) on the concentrations of ammonia and amino nitrogen in the liver of normal animals and of animals in hemorrhagic shock, indicating, particularly, that there is not any significant accumulation of ammonia This has a bearing on the problem of the differentiation between the first step, deamination, and the second, urea synthesis, and on the limiting factors in amino acid accumulation

The rise in accumulation of free amino nitrogen in these experiments also was not particularly impressive, although Dr. Russell has some other experiments which show somewhat more definite changes (21).

As far as protein metabolism in the liver goes, the data indicate definite abnormalities, but up to this point we cannot differentiate too sharply between which of these effects are due simply to a slowing of the circulation, and which are due to hepatic anoxia per se

TABLE I

Ammonia and Amino Nitrogen of Blood and Liver of Rats in Shock

	No of Obs	Blood Ammonia Nitrogen	Blood Amino Nitrogen	Liver Ammonia Nitrogen			Liver Amino Nitrogen
		mg per cent	*mg per cent increase*	*mg per cent** Free	Total	"Amide"	*mg. per cent**
Normal rats	7	0 05	0	2.5 (1 8–4 0)	9.8 (7–16)	7 3 (4 6–12 1)	55 (47–62)
Rats in hemorrhagic shock	9	0 56 (0 30–0 81)	+7 9 (+3 0–+12 1)	4 0 (2 8–5 3)	8 1† (5–16)	4.1† (1 5–13 2)	66 (57–74)

*Based on wet weight
† These averages include one very high value

Reprinted, by permission, from Wilhelmi, A. E, *et al* · Some aspects of the nitrogen metabolism of liver tissue from rats in hemorrhagic shock *Am J Physiol* **144**, *674 (1945)*

specific circulatory pattern manifested by animals that are going on to progressive failure If they do not reverse, they may be potentially significant for the progression of the syndrome There are dichotomies of functional activities, and our concern is the specificity of the phenomena that continue to persist after temporary restoration of blood volume and blood pressure, to the sequence of progressive circulatory collapse I would guess that there is forcibly reduced blood flow in the liver in many human diseases, not clear down to the shock level but low enough so that this might be treated as a common factor in various circumstances I would beg that we keep in mind two variable problems. the forced reduction in blood flow through the liver, and the interference with the hepatic enzyme systems and whether the enzyme systems recover

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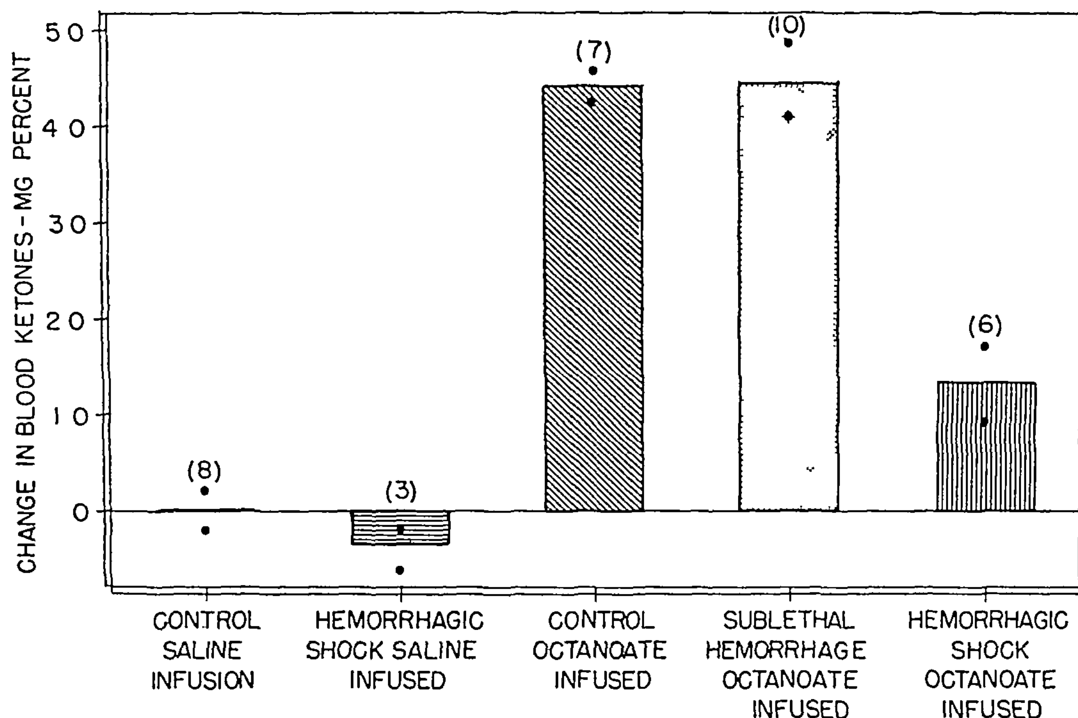


FIGURE 15 Increase in blood ketone levels after sodium octanoate infusion during sublethal hemorrhage and hemorrhagic shock compared with normal controls

done on ketone utilization, we do not have the right to say that we are measuring only ketone production

On the far left of Figure 15 is seen the effect of a 30 minute saline infusion on the blood ketones This control procedure had no effect on the ketone levels of the shocked animal Actually, a small decrease in ketone bodies resulted, similar to that in the untreated shocked animal In the normal animal, at the end of a 30-minute infusion, there was a mean rise of the order of 5 mg per hundred milliliters Animals that were bled two per cent of their body weight in an hour, exhibited a suppression of fasting ketosis but did not have an elevation in plasma amino nitrogen However, we found these rats to be quite good at making ketones after infusion of octanoate On the other hand, the animals in late hemorrhagic shock with elevated amino nitrogen levels exhibited a significant depression in their ability to form ketones from infused octanoate Whether this is wholly a problem in hepatic production of ketones, or whether there may be, as well, an increased utilization of ketones by muscle, particularly during anoxia, we are not in a position to say I suspect the former is the most important factor, but the latter may also play a role

When other metabolites are measured in the liver, one change that is prominent is the decrease in the liver glycogen that occurs in all types of shock The easiest explanation for the initial change in glycogen is epinephrine discharge, but I am sure this is not the only answer The liver glycogen goes very low, and as Dr Haist showed, is not readily restored by administration of glucose, or fructose, after animals have been subjected to shock Nor does insulin, in his experience, have a particularly beneficial effect on restoring the liver glycogen (22).

The only data that we have on carbohydrate intermediaries in the liver during shock are those published by Potter's group at the University of Wisconsin (23,24,25) They presented a spectrum of changes in various carbohydrate intermediaries, adenosine triphosphate, adenosine diphosphate, and other phosphate stores, including creatine phosphate, which showed that in hemorrhagic, drum shock, and tourniquet shock, there was a very marked depletion of all the carbohydrate intermediaries, and particularly of the high-energy phosphate compounds.

Haist In tourniquet-shocked animals, in our experience, there was a marked depletion in high-energy phosphate compounds in the clamped limbs, but even rather late in the development of shock, the forelimb muscle, remote from the site of injury, showed little, if any, depletion in adenosine triphosphate and phosphocreatin, and no reduction in the uptake of radioactive phosphorus.

Engel But in the liver, changes do occur earlier In the area of fat metabolism, very little study has been done As far as the liver goes, theoretically this should be important in terms of metabolic activity, because a high proportion of the oxygen consumption is arising from fatty acid catabolism and ketone body formation in the fasting liver

We have recently carried out some studies, shown in Figure 15, on the effects of a 30-minute infusion of two per cent sodium octanoate on the accumulation of ketone bodies in the blood during the period of infusion in normal animals, and in animals after sublethal hemorrhage and hemorrhagic shock. We found that the conversion of sodium octanoate into ketone bodies by the normal animal occurs very rapidly. The maximum increase in ketone bodies in the blood is detectable at the end of infusion, and once the infusion is stopped, ketone levels fall very rapidly to the original levels In the rat, they are back to normal 30 minutes after the infusion This technique represents a fairly sensitive measure of changes in ketone body production However, until studies are

nitrogen in the blood of rats whose entire gastrointestinal tract had been removed and the liver circulation completely shut off (the so-called eviscerated rat), as compared with rats whose hepatic artery was left intact but in which the circulation through the hepatic artery could be occluded for any given period of time. In the eviscerated rat, which is equivalent to the hepatectomized rat, there is a steady accumulation of amino nitrogen in the blood, which presumably comes from the peripheral tissues and represents amino acids which are not deaminated or assimilated by the liver. The white bars in Figure 16 represent a rise in amino nitrogen that would be expected in the totally hepatectomized animal during the time of study, while the black bars represent the rise in amino nitrogen in animals whose hepatic arteries had been occluded for the period noted, i.e, 15 to 120 minutes. Whole blood amino nitrogen levels were measured at one hour intervals during the course of the study.

Loewi. Were the rats eviscerated and only the artery kept open?

Engel. Only the artery was there In rats prepared this way, without clamping the artery, blood amino nitrogen stayed quite normal for as long as we measured it The liver with only hepatic arterial circulation had a good capacity to clear amino acids under normal conditions When the artery was occluded for 15 to 60 minutes, there was a small rise in amino nitrogen, but one not comparable to that in the liverless rat Of course, during the time the clamp was in place, the rise in amino nitrogen was comparable to that of a totally eviscerated animal because the circulation was cut off The point of interest is the recovery period after removal of the clamp Note that even with 120 minutes of total anoxia to the rat liver, the liver was quite effective in taking out the amino acids which accumulated during the clamping, and hence the liver must function quite well despite previous anoxia This supports the point which Dr Fine mentioned before that while there are *in vitro* changes indicating impaired deamination, actually the impairment in deamination is certainly less than if there were no liver

Corroborative support of this is found in Figure 17, which illustrates studies by the same technique of the respiration of the liver slice in this preparation (26) Control observations of the oxygen consumption of liver slices from animals on which this operative procedure had been performed are charted in the top line No clamp was put on the artery, and samples of liver slices were taken for Qo_2 measurement at 1, 2, 3, and 4 hours after the operation There was a slight fall during the first hour, which may

Burton: May I betray my ignorance and ask what sodium octanoate is? Is it something from which ketones are made in the body?

Engel. It is an 8-carbon fatty acid which is presumably broken down to four 2-carbon acetate fragments These condense to 2-acetoacetate molecules if ketones are going to be made from them

EFFECTS OF REDUCED BLOOD FLOW UPON HEPATIC FUNCTION

Concerning the question Dr Moe asked a few minutes ago, with regard to which of the metabolic effects under consideration are due to hepatic anoxia per se and which are due to slowed circulation, we have a few data In the experiment shown in Figure 16, a study was made of the rate of increase in amino

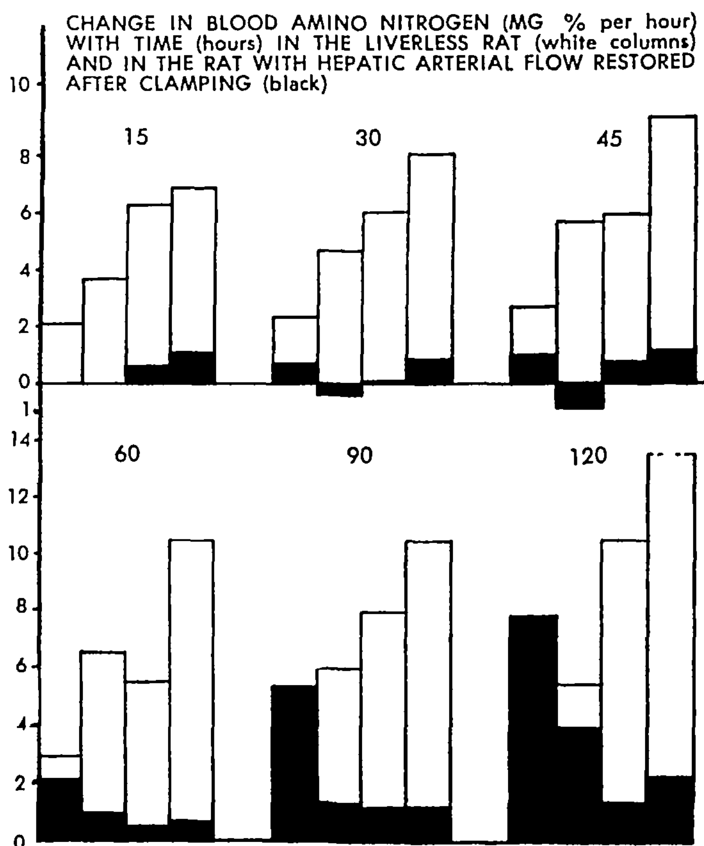


FIGURE 16 Rate of increase of blood amino nitrogen following periods of hepatic anoxia up 120 minutes in length (black columns) compared with the increases observed in the corresponding times in the liverless rat Reprinted, by permission, from Wilhelmi, A E, and Long, C N H Metabolic changes associated with hemorrhage *Ann New York Acad Sc* **49**, *605 (1948)* Calculated from data of Engel, F L, Harrison, H C, and Long, C N H Biochemical studies on shock, the role of the liver and the hepatic circulation in the metabolic changes during hemorrhagic shock in the rat and the cat *J Exper Med* **79**, *9 (1944)*

as well as it did in the previous experiment in which clearance of amino nitrogen from the blood was the criterion. While no breakdown was made of the composition of the respiration, it again suggests that the liver definitely was damaged by prolonged anoxia, but that, even so, it had a surprising capacity to recover after this treatment

Shorr. While these are comparative results and, therefore, have validity on a comparative basis, we must be aware of the fact that these may not represent the actual status of a recovering intact liver. Do I recollect correctly that when the Wilhelmis removed kidney tissue after lethal hemorrhagic shock, they found the oxygen consumption was minimally altered as compared to normal?

Engel· That is right.

Shorr: But in a normal kidney, sliced and incubated for an hour under anoxia, and then restored to oxidative conditions, there is a very profound fall in the oxygen consumption If it is left intact, not bathed in an artificial medium during the period of anaerobic incubation, the subsequently measured oxygen consumption is very much higher The very process of placing a traumatized, vulnerable slice into an artificial medium results, apparently, in considerable leaching, and effects may be produced and exaggerated so that they are not comparable to those which occur in the body in an intact organ The liver, *in vivo*, may be in a very much better position to recover, since it has not been exposed to the inevitable trauma of the sectioning and to the leaching process and dissolution of cells. Although cells in an artificial medium are rendered incapable of resisting excessive leaching by a previous period of anaerobiosis, they may be much less affected by anoxia in the body.

Engel I think that was borne out, as shown in Figure 17, with at least the one parameter of clearing amino acids from the blood. We had the clamp on for two hours and the liver was still doing very well.

Moe· I wonder whether Dr Selkurt would make a comparison of this work with the time course of recovery of renal function after total occlusion of the renal artery

Selkurt I personally have never occluded the renal circulation for this long a time Based on the work of others, it would seem that the kidney does recover rather rapidly after short intervals and even surprisingly fast after longer periods

I cannot help but make one other comment the liver, even with its high rate of oxygen consumption, can also survive this long without residual effect I believe there is probably a faster rate

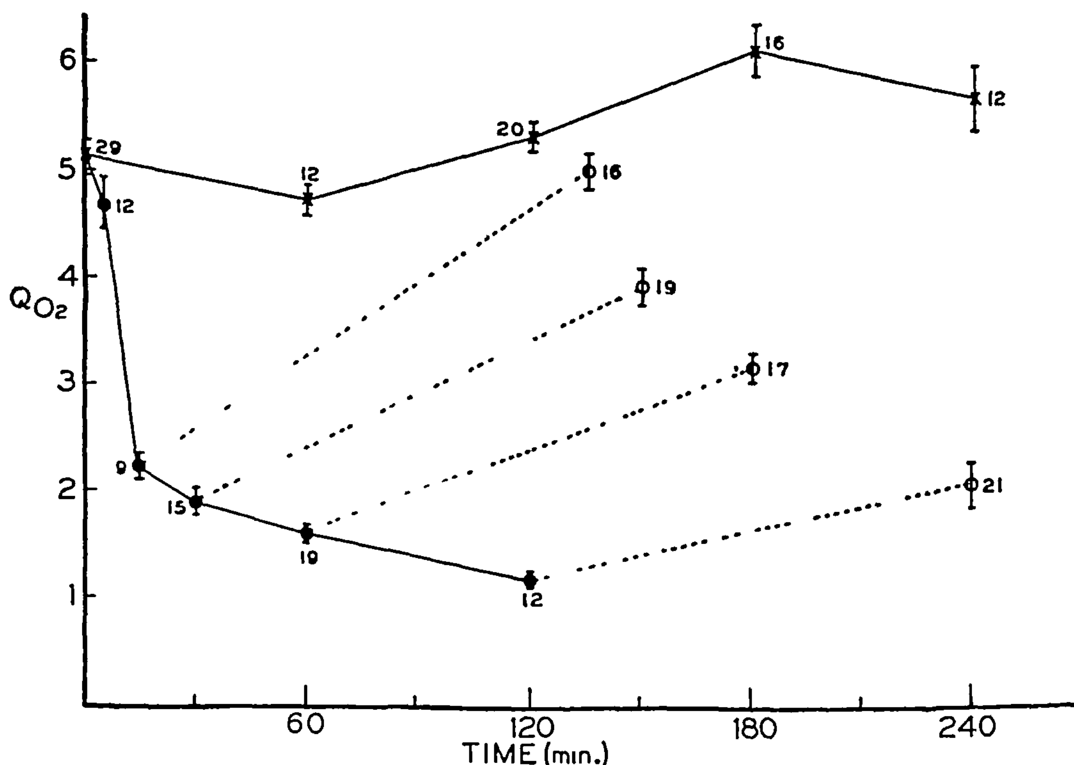


FIGURE 17 Effect of hepatic anoxia *in vivo* on respiration of rat liver slices *in vitro* Crosses control observations on liver samples from operated animals Solid circles samples from livers with hepatic artery clamped for 0, 5, 15, 30, 60, and 120 minutes Open circles samples taken 2 hours after restoration of circulation to the liver following 15, 30, 60, or 120 minutes of hepatic anoxia Small figures at each point indicate number of observations Vertical bars indicate standard errors of the mean Reprinted, by permission, from Wilhelmi, A E, Russell, J A, Engel, F L, and Long, C N H The effects of hepatic anoxia on the respiration of liver slices *in vitro* *Am J Physiol* **144**, *669 (1945)*

be related to changes in circulation from the operation itself But afterwards, the respiration recovered Actually, it was significantly higher, which is a point for speculation, this was a liver that was getting blood more highly saturated with oxygen than does the normal liver.

In other groups, the slices were removed at 15 minutes, 30 minutes, and up to 2 hours after the clamp was applied With progressive duration of anoxia, the respiration was reduced to quite low levels and did not recover when the slice was shaken in a Warburg apparatus in an atmosphere of oxygen On the other hand, when the clamp was removed at various intervals and the circulation was restored for 3 to 4 hours, minus the period that the clamp was on, and then the Q_{O_2} measured, there was still fairly good recovery, except when the artery had been occluded for 1 to 2 hours. In this function, however, the liver did not perform

three per cent of the body weight. We realized that this situation was not "shock," although it may be cogently argued that the changes and compensatory adjustments involved did not differ greatly from those occurring early in shock

Estimated hepatic blood flow (EHBF) was measured in nine dogs before and after hemorrhage In nine animals, hepatic arteriovenous oxygen difference was determined, and in four the renal blood flow was measured The Bromsulfalein (BSP) method (28) was employed for the determination of EHBF. For this purpose a catheter was inserted deep into a hepatic vein by way of the internal jugular, under fluoroscopic control Bromsulfalein was administered intravenously at a constant rate ranging from 1.1 to 2 3 mg per minute in different animals, and the blood level maintained as nearly constant as possible between 1 and 2 mg per cent Arterial blood was taken at approximately 10-minute intervals and hepatic venous samples obtained at similar intervals, the rate of

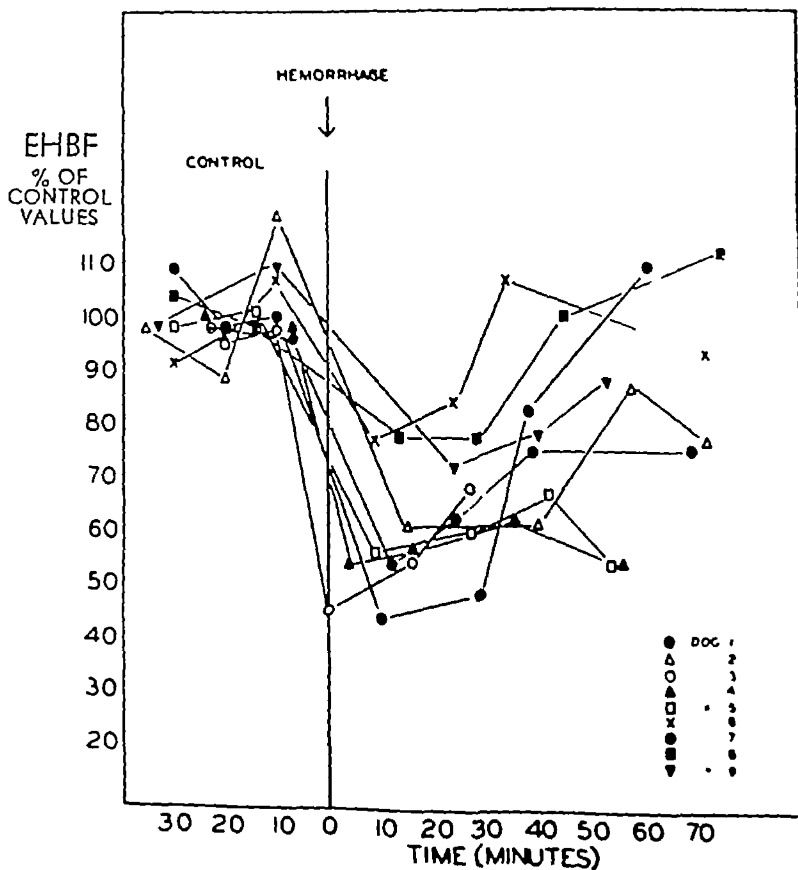


FIGURE 18 The effect of hemorrhage on estimated hepatic blood flow in the dog Reprinted, by permission, from Heinemann, H, Smythe, C, and Marks, P The effect of hemorrhage on estimated hepatic blood flow and renal blood flow in dogs *Am J Physiol* (In press)

of oxygen consumption in the liver than kidney.

Bradley: The liver has a higher rate of oxygen consumption per unit mass of tissue.

Selkurt: I suppose there is a rough correlation in their survival The recovery here after two hours of blocking is not too different from the kidney, which also survives and recovers after roughly that same period of time.

Bradley The kidney can survive a prolonged period of complete ischemia in man during the operation of splenorenal anastomosis The renal artery may be occluded in dogs for as long as two hours without permanent damage (27)

Knisely Right

Shorr. The critical period was two to four hours.

Bradley I don't know how long cellular function is reduced after such a period of anoxia But I believe work in animals indicates that the extraction of sodium *p*-aminohippurate is impaired for a considerable period after arterial occlusion lasting two to four hours (27)

Cotzias Yes.

Bradley· The term "recovery" can have a variety of meanings. It may mean recovery in terms of return of blood flow to normal, or it may mean recovery of intrinsic cellular processes I would be loath to use the word as a general term

HEPATIC BLOOD FLOW DURING HEMORRHAGE AND SHOCK

Shorr. I wonder whether it would be appropriate at this point for Dr Bradley to discuss some of his findings on liver blood flow following hemorrhage

Engel Yes, this would be an appropriate point

Bradley The study I wish to discuss with you was carried out by Drs Heinemann, Smythe, and Marks in my laboratory during the past year We have been interested in the general question of the contributions of the various discrete circulations to systemic hemodynamic adjustments Since hepatic blood flow does not differ greatly from the renal blood flow it seemed likely that the splanchnic vasculature might participate as prominently in these responses as the renal vasculature Moreover, Dr Smythe and I had observed instances of excessive reduction in estimated hepatic blood flow in dogs during alcohol narcosis It was decided to subject dogs to a relatively mild, but effective, and standardized "stress," i e, rapid loss of blood in amounts ranging from two to

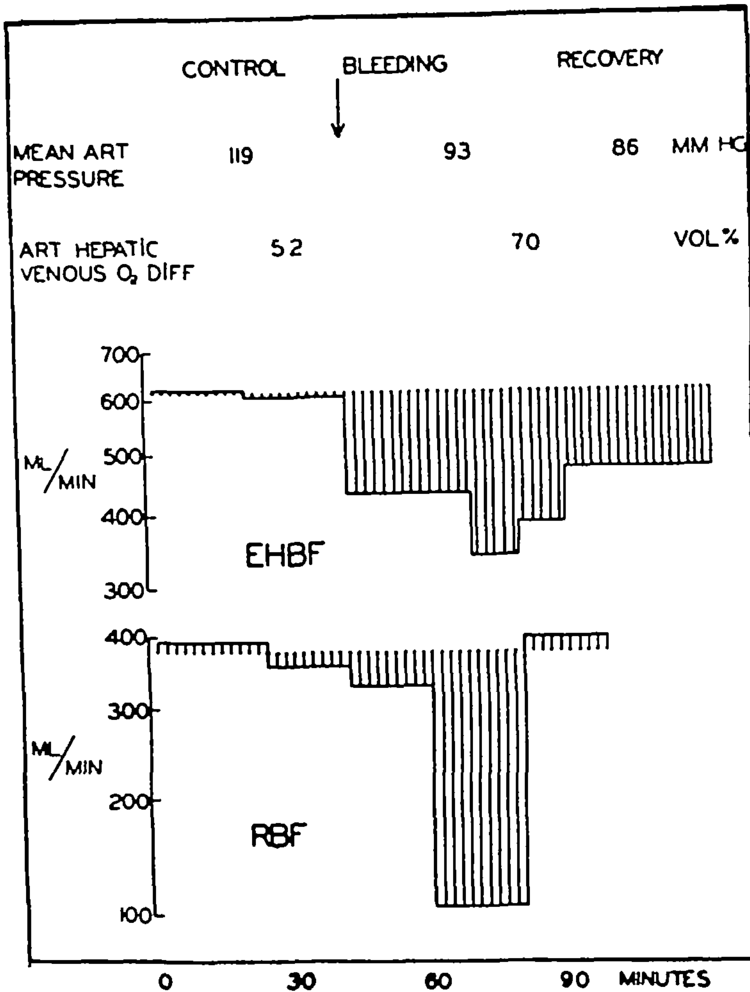


FIGURE 19 The effect of hemorrhage on mean arterial pressure, hepatic arterio-venous oxygen difference, hepatic blood flow (EHBF) and renal blood flow (RBF) in the dog Reprinted, by permission, from Heinermann, H, Smythe, C, and Marks, P The effect of hemorrhage on estimated hepatic blood flow and renal blood flow in dogs *Am J Physiol* (In press)

also increased when EHBF fell Statistical analysis indicates that these changes are highly significant

It may be concluded that the splanchnic circulation plays an important role in the hemodynamic readjustments following hemorrhage As in the kidney, hepatic vasoconstriction serves in the maintenance of arterial pressure and supplements the cardiac output by diverting blood from the splanchnic bed to other areas more susceptible to the ill-effects of hypotension

Stead Were these anesthetized or unanesthetized animals?

Bradley Sodium nembutal was given intravenously in doses of 25 to 30 mg per kilogram of body weight prior to each experiment

BSP removal was taken as the rate of infusion at constant blood levels of BSP, and this value divided by the arteriovenous BSP plasma concentration difference yielded a value for hepatic plasma flow. Oxygen was measured in arterial and hepatic venous blood, obtained anaerobically before and after hemorrhage in nine dogs by the method of Van Slyke and Neill (29) The renal plasma flow was determined as the para-aminohippurate clearance Arterial hematocrits were used for the calculation of blood flow from the values for plasma flow

In Figure 18 all values for EHBF obtained before and after hemorrhage are plotted as percentages of the averaged control values against time It can be seen the EHBF invariably decreased after the hemorrhage Since the arterial pressure decreased very little as a rule, the decrement in blood flow is attributable to increased resistance in the splanchnic bed, presumably as the result of active vasoconstriction It is particularly interesting that EHBF tended to return toward the control level after approximately twenty minutes This recovery in EHBF occurred in the absence of any significant change in the arterial pressure The hematocrit tended to fall by this time, and it is possible that the spontaneous recovery in EHBF reflects the tendency of plasma volume to recover

Shorr Could you tell us the range of the hypotension?

Bradley The mean arterial pressure decreased 5 to 50 per cent and did not fall below 60 mm Hg

The renal blood flow (RBF) decreased following hemorrhage in company with EHBF, as the experiment presented in Figure 19 demonstrates, and it decreased in every instance to a greater extent than EHBF We were somewhat surprised to find that RBF also recovered spontaneously without change in arterial pressure. Thus vasodilation sufficient to bring the blood flow back to control levels occurred in both the splanchnic and renal vascular bed Calculation of resistance revealed a fall at this time below control levels This response suggests that a state resembling "reactive hyperemia" in its basic hemodynamic pattern may develop in the kidney and liver after a period of acute vasoconstrictive ischemia Further investigation of this phenomenon is necessary to establish its mechanism.

The hepatic arteriovenous oxygen difference rose (Figure 19) during the period of ischemia following hemorrhage in proportion to the decrement in EHBF, indicating that splanchnic oxygen consumption was not greatly affected Bromsulfalein extraction

hepatic removal. Probably the most serious error arises in sampling blood from one hepatic vein, mixed hepatic venous blood being unobtainable. We have found that determinations of EHBF in different lobes of the liver differ no more than values of EHBF determined in a single lobe. Pratt, Burdick and Holmes (32) have also found that left hepatic venous and mixed hepatic venous plasma BSP concentration do not differ significantly in the dog.

Haist: Would a change in liver function alter the validity of the procedure?

Bradley. A great deal depends upon the extent to which BSP removal and extraction are depressed. It may be assumed that extra-hepatic removal is not greatly altered, and if so, taking the figures I've already mentioned, extra-hepatic removal makes up an increasing proportion of the total removed as hepatic removal is reduced. Thus with a removal rate of 0.5 mg. per minute, the extra-hepatic removal of 0.04 mg. per minute makes up eight per cent of the calculated rate, and the error may be even larger. Of more importance is the change in extraction and the limitations of the analytical method by which BSP is determined. When arterial and hepatic venous concentrations differ by 10 per cent or less, at or below 1 mg. per 100 gm. the analytical error bulks large and invalidates the estimation of blood flow.

Burch. Has anyone measured blood flow directly and correlated it with the clearance studies?

Bradley: No one has.

Burch: Is this also true for the kidney?

Bradley: Blood flow through the kidney has now been measured directly and compared with PAH clearance.

Green: If you feel that the Bromsulfalein method is that good, why are you afraid to use it at lower extractions?

Bradley: Because I don't believe the method is valid when the extraction is less than we can measure accurately by the chemical method we use at present. In the studies presented here the extraction actually increased and this difficulty never arose. Possibly when we examine animals in severe shock our fears will prove groundless for the same reason.

Burton: Does the method give blood flow changes in absolute or relative figures?

Bradley: It gives an absolute value.

Burton: It is not giving blood flow per gram of active tissue?

Shorr: If we relate these degrees of hemorrhage to the shock syndrome as we ordinarily produce it in our laboratory, then the changes that you describe are associated with what we call the hyperreactive phase. This phase is characterized by recovery on transfusion, and is associated, from our indices and those of Van Slyke and others, with the persistence of oxidative metabolism throughout the liver.

Bradley: I should stress the fact that recovery occurred without treatment of any kind. There was a fall in the hematocrit and the blood pressure changed very little

Fremont-Smith: Since the circulation returned both to the liver and to the kidney, you might say that the fact that the blood pressure did not recover was perhaps related to the vasodilation, or the return to full circulation through the liver and kidney

Bradley: Vasoconstriction apparently played a role in maintaining the level of blood pressure

Fremont-Smith. I am just saying the same facts in a slightly different connotation

Shorr Of course, what we are waiting for you to do is to continue this in the more severe, refractory, or irreversible stages of shock

Bradley I am rather afraid that the BSP method may prove invalid in severe shock owing to inadequate BSP removal Perhaps I am unduly pessimistic

Shorr I gather then that oxidative processes must be essential for adequate extraction of BSP

Haist How valid is the method for blood flow estimation?

Bradley The validity of the major assumptions on which the method is based is now fairly well established The error involved appears to be of the same order of magnitude as that in measurements of renal blood flow by the PAH clearance Extra-hepatic removal of BSP at the plasma levels employed in measuring EHBF amounts at most to about 5 per cent of the total removal rate Werner and Horvath (30) have found that approximately 30 per cent of the BSP in the blood is removed in an hour in the eviscerated animal Assuming a plasma level of 1 mg per 100 gm and a plasma volume of 800 ml, then 30 per cent of 3 mg, or 2 4 mg is removed in 60 minutes or 0 04 mg. per minute In the intact animal, BSP removal at plasma levels of 1 mg per cent amounts to about 2 mg per minute Extra-hepatic removal thus accounts for 0 04 of the 2 0 mg removed per minute, or 2 per cent, and may therefore be considered a negligible source of error Dr A Macpherson and I (31) have obtained a similar figure for extra-

hepatic removal Probably the most serious error arises in sampling blood from one hepatic vein, mixed hepatic venous blood being unobtainable. We have found that determinations of EHBF in different lobes of the liver differ no more than values of EHBF determined in a single lobe Pratt, Burdick and Holmes (32) have also found that left hepatic venous and mixed hepatic venous plasma BSP concentration do not differ significantly in the dog

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Burton Does the method give blood flow changes in absolute or relative figures?

Bradley It gives an absolute value

Burton It is not giving blood flow per gram of active tissue?

Bradley: No. Moreover, we think it measures hepatic blood flow in particular because we have no evidence of significant BSP removal elsewhere in the splanchnic bed.

Engel. It might be worth-while to discuss what, if anything, these metabolic changes in the liver mean. I don't think I can give an answer but I should like to point out certain things: while it is very nice to have a relatively good correlation of certain phenomena occurring in the shocked liver with certain changes after clamping off the circulation to the liver, it is important to realize that these two processes are by no means equivalent. In the shocked animal, there are presumably all sorts of metabolites circulating about which are coming from hypoxic tissue elsewhere. Combined with a slow circulation through the liver and a low supply of oxygen, these metabolites might do something very different to the liver, both immediately and in terms of its ability to recover, from simply cutting off the circulation for a given period of time and then letting good, healthy blood flow back through it. I certainly don't think one can say, on the basis of any data existing so far, that it has been proved that hepatic failure is the cause of the final breakdown and production of so-called irreversible shock, or even of death itself. I think the closest that any work comes to it is that of Dr. Shorr on VDM inactivation, which might be considered as an example of liver failure which is critical in terms of homeostasis.

Burch: I should like to ask two questions. Has any liver function test been found which is not altered in shock? Has there ever been any qualitative disturbance in function which is peculiar to shock and not found in other physiologic states?

Engel: Offhand, I can't think of any liver function which has been tested adequately that hasn't been found disturbed, but I am not sure that all liver functions have been tested. And as to whether any of these changes are more specific to shock, I think in the gross sense they are not, in other respects they may be, for the degrees of severe disturbance in metabolic function which occur in shock are practically never seen in ordinary types of liver disease. They appear very late in fulminating liver disease, but they are much more striking in most cases of shock.

Burch: Then the changes are quantitative rather than qualitative?

Engel: I think so.

Nickerson: The question in my mind is whether the metabolic changes which have been measured are actually part of the chain of cause and effect which finally leads to the death of an animal, or whether they are merely measurements which tell us that some-

thing is wrong but which are not etiologically involved in the shock process. Are they the same as the cold, clammy skin of the patient in shock, which tells us that something is wrong but certainly does not kill the patient? Your last statements suggest that you feel these are simply symptoms, like the cold, clammy skin.

Engel. Your point is well taken, I think that is the critical aspect of the whole problem. However, we have very few data that enable us to answer whether "cold, clammy liver" is a symptom like the "cold, clammy hand." If it is, we aren't going to learn very much by further work along these lines On the other hand, if the "cold, clammy liver" is affecting the whole circulation, then it is proper to pursue this further

Nickerson. The reason I raise this point is that these data as such are not really points for discussion or argument, they are generally accepted I am trying to get you to stick your neck out, on the possibility that there would be a real difference of opinion as to what these data mean

Engel. This is where we could use some new bottles to pour the old wine into

Nelson I, too, agree that Dr Engel has painted a beautiful picture of the metabolic pathways in shock Whether these derangements are etiological or secondary to diminished flow and hypoxia is the question I think the connotation that Dr Engel would like to leave with us might be illustrated by an analogy. As the seasons change from fall to winter, we observe that the leaves on the trees turn from green to orange, red, or yellow, varying with the species of the tree and the particular biochemical reactions involved No matter how critically we measure the spectrum emitted by the colors of these leaves, it represents a description of variations due to change in the season, and does not indicate that these variations in color are *causing* the change from autumn to winter

Shorr That is an excellent analogy

Engel I wonder whether Dr Fine would want to say something about his experiments on irreversible shock in which recovery resulted after infusion of arterial blood into the portal vein At the time those experiments were reported, I felt they were very good evidence that perhaps the failure of the circulation through the liver, and its restoration by blood high in oxygen, really meant something with regard to the importance of the liver in shock

Fine I still feel it means something, although at the time we tried to interpret its meaning we were not able to say from our results whether the unprotected liver was manufacturing some kind

of lethal substance, or whether we were protecting the liver so that it could continue to function normally. The former view was the more appropriate implication from Dr. Shorr's VDM data. From our data, we were not able to draw the definitive conclusion that he drew from his. But today I should confidently answer that we were dealing with both factors. (*a*) a toxic substance developing in the liver, and (*b*) maintenance of normal liver function, particularly its detoxifying function But it is the liver of the dog in every case, and I think we should bear that in mind in view of our subsequent studies with antibiotics and the differences in the bacterial content of the liver in different species Our data do not necessarily apply to the liver of other species.

Knisely. There are several sets of circulatory functions of the liver, and many of the things just said fit in very closely with general knowledge The liver is a large blood reservoir The circulation through the liver, of necessity, nourishes the liver. The liver contains the major part in the body of the phagocytes, which can remove particles from the blood, so that it is always tied up with defense mechanisms. One of the first responses to hemorrhage in animals consists of very specific, detailed vasomotor responses, wherein the liver empties out blood which it contains and parts of the vascular system shut off so that no more blood can come in (33)

One thing we are very much interested in is how long, as a part of normal physiology, can a portion of the liver have its blood supply shut off before the tissue of the liver is damaged? This is important because in order to carry out the necessary vasomotor responses to maintain circulation, parts of the liver sometimes are shut off without having any blood in the sinusoids, and at other times, large amounts of blood are stored in the sinusoids, with that blood standing still Up to a certain time, hepatic tissues can recover Beyond that point, I should almost guess that they have to fail. What is the nature of that time? How long is it in relation to other things?

I should like to mention the fact that if the circulation is shut off through the liver for a long enough time, any bacteria in the circulation are prevented from getting to the hepatic phagocytes, and, because the blood from the spleen must pass through the liver, blood is necessarily prevented from coming into the spleen Therefore, two of the major phagocytic defense mechanisms have been thown out of gear

Fine. We have demonstrated blockade in the liver by producing plethora in the animal in the irreversible state The blockade is so

severe that there is a hemoperitoneum as well as intestinal bleeding We have found as much as 2000 or 3000 ml of what looked almost like whole blood and we made sure it was not due to a technical surgical error Dr. Bradley's data do not apply to the state I am talking about, namely, well-developed hemorrhagic shock When we visualize the venous system in the liver in this situation, we find a widespread venous constriction, simultaneous with an absolute increase in portal pressure on giving transfusions However, blockade is not a phenomenon related directly to irreversibility, because an Eck fistula does not alter the general course of events in shock.

Burton I wonder how Dr Nickerson got away with his remark about "cold, clammy skin." He said that was not what was killing the patient. I thought Dr Nickerson was one of the people who has shown that in a sympathectomized animal a cold, clammy skin makes a tremendous difference as far as the survival of the animal is concerned It seems to me that the entire discussion was rather loose, because we were told also that maybe a "cold, clammy liver" did this If the blood supply of the liver is cut off, the liver is not cold It remains about the same temperature, or it drops, perhaps, a quarter of a degree Maybe Dr. Nickerson's remark was facetious

Shorr. That is the trouble with having a physicist in our midst

Burton I do not think it gets clammy, either

Knisely That is important The experiment gets cold, but the liver is a beautifully incubated lesion at that time The liver is kept warm by the rest of the body

Nickerson Perhaps I should explain more fully what I meant The cold, clammy skin I referred to is what the clinician sees and feels The fact that it is cold and clammy is an indication that there is a peripheral vasoconstriction It is simply a measure, as the absorption of light in a colorimeter is a measure, of something else that is going on The fact that it is cold and clammy, I am sure, is not producing the damage, but the vasoconstriction which induced the coldness and clamminess, as well as many other changes, may be important in the etiology of shock

I wanted to raise the question of a distinction between a factor such as vasoconstriction, which may be etiologically related to the whole course of events leading ultimately to death, and something such as optical paleness, which is a measure of the constriction but is not per se a factor in the development of subsequent lesions I think that the critical point in this whole problem of the metabolic changes in shock is whether they are simply things which we can

measure, and which tell us that something else is going on, or whether they are integral parts of the chain of events leading to death.

Shorr. To show further the dangers of analogy, the cold clammy skin may be one of the important phenomena preserving the animal and the patient

Knisely: I want to comment once more on some of Dr. Engel's work. It is not yet common knowledge in the textbooks of medicine, but in monographs on the control of blood flow through the liver there are evidences to the effect that the hepatic artery can be shut off with the portal vein circulation running rapidly for long periods, and vice versa (34)

RELATION OF HEPATIC BLOOD FLOW TO VDM PRODUCTION

Shorr What we are all looking for is something in the nature of a specific metabolic defect which can be related to the sequence of events occurring during the progression of the shock syndrome to final circulatory deterioration I should like to present a little of the evidence gathered in our laboratory, with Doctors Zweifach, Baez, Furchgott and Mazur, which we believe represents at least a step towards that objective It is an approximation that I am sure will only be complete when we relate it to the exciting phenomena which Dr Fine and his laboratory have uncovered during the past two years

The two factors that we are most concerned with (for simplicity's sake I exclude the muscle vasodepressor) are the hepatic vasodepressor, VDM (ferritin), which is a normal product of anaerobic or hypoxic metabolism of the liver, and which, under oxidative conditions, is acted upon by the liver to render it vasoinert, and the renal vasoexcitor, VEM, which is likewise a product of hypoxic or anaerobic metabolism of the kidney, and which, in comparable fashion, is rendered vasoinert by the aerobic kidney. As Dr Zweifach told you last year, these substances are peculiar in that they affect the behavior of the terminal vascular bed, one by depressing, and the other by potentiating the response to topical epinephrine of the muscular vessels of the capillary bed

If we look at this semidiagrammatic chart (Figure 20) of the behavior of a dog who is first exposed under sodium pentobarbital anesthesia to a period of moderate hypotension, produced by moderate bleeding, and then to a period of more drastic hypotension for, let us say 90-120 minutes, we see that a very definite sequence of events occurs There are first of all the vascular changes which Dr Zweifach described at our last meeting, the development

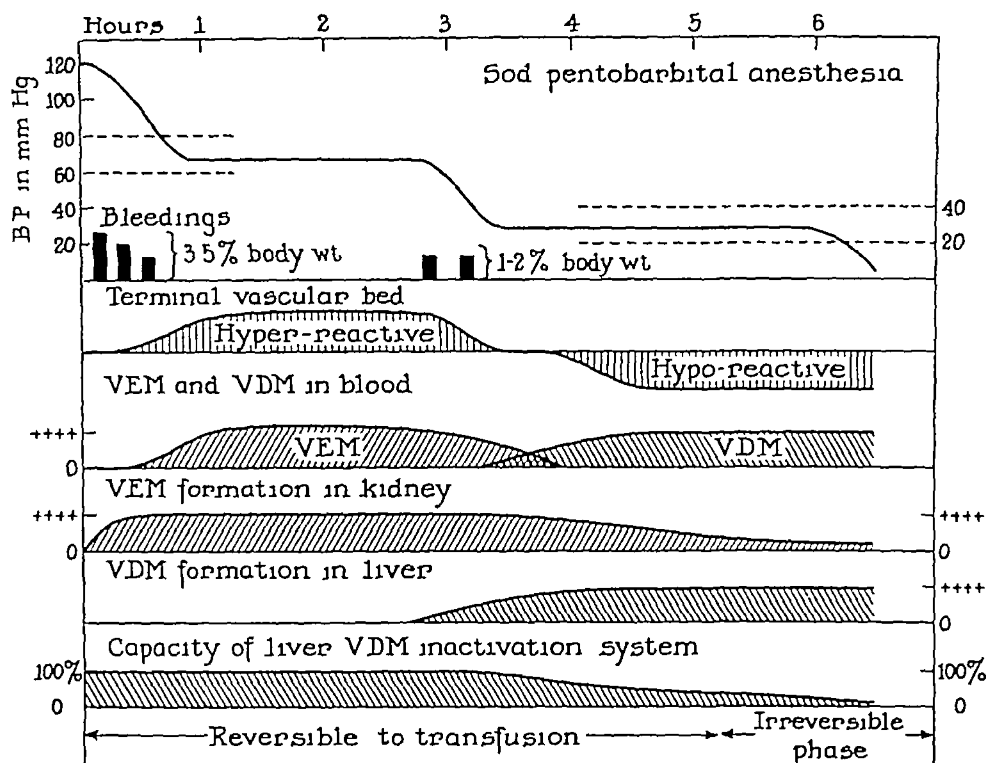


FIGURE 20 Schematized protocol of sequence of vascular and humoral events following graded hemorrhage Compensatory response of capillary bed (hyper-reactivity to topical epinephrine and augmentation of vasomotion) is associated with elaboration of VEM into circulation by kidney Capacity of kidney to form VEM becomes progressively impaired with persistence of hypotension and prolonged renal hypoxia Decompensatory vascular pattern (hyporeactivity to epinephrine and suspension of vasomotion) associated with elaboration of VDM (ferritin) into blood by hypoxic liver Continued formation of VDM by liver is related to impairment of hepatic enzyme mechanisms for inactivation of vasodepressor Irreversible phase of syndrome characterized by vascular decompensation and deterioration of VDM mechanisms in liver Reprinted, by permission from Shorr, E , Zweifach, B W , and Furchgott, R F *Research in Medical Science*, Green D and Knox, W E , Editors, New York, Macmillan, 1950 (p 323)

of vascular hyperreactivity, with an ischemic type of flow, and with profound increases in the reactivity of these terminal vessels, and then, with the advancement of the syndrome through the progressive hypotensive states there are reversions to capillary hyporeactivity and hyperemia Chronologically, these changes are associated with the appearance in the blood of the two humoral factors, described above VEM of renal origin appears first, and subsequently VDM, of hepatic origin During the period of moderate hypotension, the blood flow to the kidney is sufficiently reduced to shift its metabolism over to the anaerobic type, with the release of the vasoexcitor, VEM If we exclude the kidney from participa-

tion, this material does not appear in the blood, and these typical reactions do not unfold in normal fashion. During the period of moderate hypotension, the blood flow to the liver is sufficient to support aerobic processes and there is no release of the vasodepressor material Van Slyke and his associates have shown the persistence of oxidative mechanisms in the liver at moderate hypotensive levels, and the abolition at the more drastic hypotensive levels which lead eventually to irreversibility (35)

Throughout the syndrome, the vasoexcitor material continues to be formed in the kidney, but by the time the blood pressure has fallen to the drastic hypotensive levels of the phase that eventually results in refractoriness to transfusion, the renal blood flow has fallen virtually to zero, and the vasoexcitor is no longer capable of entering the circulation By this time the liver has shifted to an anaerobic type of metabolism and releases the vasodepressor material, ferritin If this continues long enough – let us say, 90-120 minutes – the animal becomes refractory to retransfusion of the blood lost, and we observe a concurrent change in the liver system which normally renders this material vasoinert In other words, the ferritin inactivation system progressively deteriorates Finally, if one excises the liver at this stage of shock, and incubates slices under oxidative conditions, one can demonstrate that vasoactive ferritin continues to be released despite these aerobic conditions, and when that occurs the animal proves unresponsive to transfusions

Fremont-Smith You mean, at that point, the liver continues to make ferritin, even though oxygen has been restored to it?

Shorr Yes, even though there has been a restoration of oxidative conditions

Richards: During the VEM phase, does VEM promote liver blood flow, or does it attempt to sustain it?

Shorr We cannot tell All we know is that throughout the VEM phase – and in the unanesthetized animal this phase persists right through the drastic hypotensive period – the liver does not release vasoactive VDM, and its capacity to inactivate ferritin does not suffer

Fremont-Smith That is without anesthesia?

Shorr. Yes, without anesthesia blood pressure as low as 35 mm Hg can be sustained and the animal can be kept alive for many hours with small transfusions to prevent ischemic death VEM persists in the blood and liver inactivation mechanisms are preserved.

We have been able to relate the vasoactivity of ferritin to the state of specific chemical groups in the molecule, namely, the sulfhydryl groups (36) When the sulfhydryl groups of ferritin are oxidized by copper and oxygen, or blocked, by other reagents, ferritin loses its activity When reduction is achieved as by cysteine or ascorbic acid, to the sulfhydryl state, ferritin becomes vasoactive once more The normal liver contains only disulfide ferritin If liver is incubated under anaerobic conditions, there is a progressive reduction of the disulfide to the sulfhydryl ferritin with the acquisition of vasoactivity When sulfhydryl ferritin is incubated aerobically with liver slices, oxidation to the vasoinactive, disulfide form occurs. We believe these reactions are enzymatically mediated in that they can take place in cell-free eluents of aerobic liver (37)

The evidence we have indicates that this is a normal metabolic substance Destruction of the liver is not necessary for its production and we definitely do not believe it is a toxin We believe these reactions have a place in normal physiology, with their direction shifted during the progression of the shock syndrome so that sulfhydryl ferritin is formed and not inactivated Indeed, in hypertension, with a demonstrably aerobic liver, something happens to the intermediary metabolism of ferritin, as a result of which the aerobic liver shifts the equilibrium of the reversible chemical reaction in the direction of SH-ferritin, so that vasoactive SH-ferritin is released into the blood stream continuously for as many years as the hypertension persists.

Fine· At last year's Conference, we briefly discussed the question of whether SH-ferritin is the vasodepressor substance in shock. I never quite understood your explanation for what appears to me to be an obvious discrepancy This iron-containing protein is present in other tissues, which are also exposed to the anaerobic state They, too, should show SH-ferritin, and yet it cannot be extracted from the other tissues as it can from the liver That is what makes me wonder whether it is this substance, or something else in the liver that is not to be found in other tissues, which exerts the vasodepressor action you have observed

Fremont-Smith I understand that you are not calling ferritin toxic, but that there is a toxic substance from the liver which is not ferritin? Is that correct? Or were you saying that if ferritin is coming from the liver, then it must be toxic? I could not quite make the distinction

Fine No, I do not want to put it that way. It is a question of semantics. If whatever comes out of the liver in shock is deleterious

to the animal, that makes it necessary for me to say that such a substance, if it kills, is poison It does not matter whether this substance is a natural product or not. Any substance which kills is a poison.

Fremont-Smith Insulin can be a toxin in spontaneous hypoxemia?

Fine· That's right

Shorr Ferritin is present in the normal liver cell almost exclusively (99 per cent) in the disulfide form, which is inactive as a vasodepressor, but under anaerobic conditions it is progressively converted to the sulfhydryl or active form (37) On the other hand, we have found that in the hypertensive dog liver, which is not hypoxic, 15 to 25 per cent of the ferritin is in the sulfhydryl form, showing that some change in the equilibrium between sulfhydryl and disulfide ferritin has taken place

I think perhaps we have not emphasized that in one other organ, the spleen, in which the concentrations of ferritin are large enough for us to deal with chemically, anaerobiosis does release vasoactive SH-ferritin In our first publication (38) in 1945, we said the spleen had no ferritin, but subsequently we found we were missing it because of the high concentrations which give a misleading reaction in the rat meso-appendix test When we diluted the saline washes of spleen, often as much as ten times, we found it to be present just as we had in the liver wash Hence in this tissue as well, anaerobiosis does lead to the release of ferritin in the vasoactive sulfhydryl form, just as it does in the liver However, spleen, unlike liver, cannot inactivate sulfhydryl ferritin

Fine Excuse me for sounding a little dull The SS form of the protein, I understand, is present in considerable quantity in tissues other than liver and spleen, isn't that so?

Shorr Ferritin is present in many tissues, for example, the kidney But we do not get any vasodepressor effect from the kidney under anaerobiosis, except under very unusual conditions The adrenalectomized animal will release vasoactive ferritin

Fine. From the kidney?

Shorr· Yes, from the kidney

Fine Can you explain why you cannot find this vasodepressor substance in saline extracts of other tissues? Is it a matter of the amount that they release?

Shorr· The amounts are small. With the precipitin reaction, it can be demonstrated to be present in many of the tissues of the body, but the amounts that are available would have to be reduced to SH-ferritin to become vasoactive

Fine: Aren't they reduced by the fact that they are as anoxic as the liver?

Shorr: We believe the concentrations are too small The rat test is not sensitive to highly active ferritin in amounts of much less than 0.0005γN

Fine I recall your assay of the blood coming from the vein of the tourniqueted extremity having a very high concentration

Shorr That is right

Fine: As high as anything you found in the liver?

Shorr. We do not know yet the relationship to ferritin of the vasodepressor material coming from muscle It is not inactivated by antiferritin serum although it has the same action on the terminal vascular bed as ferritin, and, like ferritin, it is inactivated by the liver However, we have not established the chemical nature of the vasodepressor material from muscle

Ferritin is an important constituent of the body, and it may be released in vasoactive form under conditions in which there is no deterioration of the liver cell In cirrhosis and hypertension the equilibrium reaction has been shifted to the anaerobic form by an unknown process which permits the release of vasoactive ferritin under aerobic conditions

Nickerson Since presumably this is not a spontaneous oxidation reduction, but requires enzymatic action, has such a system been demonstrated in other tissues Dr Shorr?

Shorr As far as we know, no other tissue will inactivate ferritin.

Nickerson Perhaps that provides at least a partial answer to Dr Fine's question Although the material may be present in other tissues, it may not be released in active form during anaerobiosis, unless the appropriate enzyme system is present

Shorr Correct We know, however, that the systems which are necessary for the release of vasoactive ferritin are present in the spleen

Green In discussion of the term "toxic," do we know of any function which this active ferritin performs in the body?

Shorr We know that it depresses the reactivity of the muscular vessels of the terminal vascular bed We know that it is a profound antidiuretic, and that it is present in antidiuretic states in man, such as decompensated cirrhosis, nephrosis and heart failure with edema We know that ferritin stimulates the neurohypophysis to the release of pitressin-like materials, which then appear in the urine

Fine. Have you done these observations on ferritin in the liver in the rat?

Shorr. These observations were made in rats, rabbits, dogs, and on human biopsy samples

Burton· Dr. Shorr, my question was asked before in our last session when we had an interesting discussion on this matter I think it was answered in the negative, but the answer may have changed Have you been able to demonstrate that the infusion of ferritin, let us say, into an animal seriously affects the course of shock? By that I do not mean that it seriously affects what you observe in the mesentery, but affects the course of shock in the animal

Shorr I cannot answer that any more clearly than the last time. It represents an important direction, which we are not pursuing as vigorously as we should, for the same reasons that none of us can pursue at any one time all of the important directions of our research We feel that per se ferritin is not a hypotensive agent For example, in nutritional cirrhosis there is a constant ferritinemia The blood volume is at least normal, and may be increased The reactivity of the peripheral vessels is depressed but the animal gets along very well despite the ferritinemia However, if this animal is exposed to a shock procedure, with a given mortality in the normal animal, that mortality is greatly enhanced, presumably because the cirrhotic animal is unable to make the necessary vascular adaptations of a compensatory sort It goes into shock and collapses, as Toby and Noble showed (39) under circumstances in which the majority of normal animals make an effective readjustment The infusion of ferritin would not be expected to affect the course of shock unless the capacity of the liver to inactivate the ferritin were overwhelmed

Burton I think this is a crucial experiment People might say, "We know that urea piles up during shock," but I think no one would admit that urea plays a determining role in the course of the shock in that animal.

Shorr. The direct demonstration that you want is not yet at hand, but later on I should like to present some indirect evidence which may have some bearing on the interpretation of our data.

REDUCED RENAL BLOODFLOW AND VEM PRODUCTION IN SHOCK

I should like to bring some evidence to bear on the significance of these factors by showing what happens when we interfere with

the full participation of the renal factor in this syndrome. With the Wiggers' type of graded hemorrhage, unanesthetized animals survive for long periods of time at very drastic hypotensive states, and unless they die of ischemic shock they are recoverable by transfusion. Throughout this whole period of drastic hypotension, VEM continues to be found in the blood stream. In other words, we believe there is sufficient renal blood flow to release VEM constantly into the circulation at blood pressure levels which, in the anesthetized animal, would completely abolish renal blood flow. The liver inactivation system remains entirely normal, and the animal is recoverable.

If we utilize the same bleeding procedure in an animal which we have previously prepared by removing one kidney and transplanting the other kidney in the flank, so that we can tie it off under procaine just before we bleed, then we find an entirely different sequence of events. In the first place, there is little or no tendency on the part of the blood pressure to rebound to higher levels after each withdrawal. In the second place, no VEM appears. In the third place, VDM, ferritin, appears extremely early in the syndrome and rapidly increases in concentration in the blood. If we take a liver biopsy, instead of 100 per cent inactivation, we find a profound deterioration in inactivating capacity. We find high concentrations of ferritin in the liver wash, and when we transfuse we find the animal has become irreversible.

Engel What is the Q_{O_2} in the liver at that point?

Shorr In our experiments the Q_{O_2} of the liver in the irreversible stage of shock averaged 85 per cent of the normal. And yet the inactivation mechanism may have deteriorated completely.

Dr Zweifach, you visualized the blood vessels after shock induced in the arenal animal. Would you describe the differences which you observed in this type of animal compared with the normal animal having the kidney intact?

Zweifach The circulatory changes in the arenal dog subjected to hemorrhage are typical of those observed in irreversible shock. Vascular compensation is inadequate. The responsiveness of the metarterioles and precapillaries to topical epinephrine, which is an excellent reflection of the functional capacity of the terminal vascular bed, shows no compensatory augmentation. In fact, vascular hyperreactivity is usually minimal or lacking in these animals. Spontaneous vasomotion becomes considerably increased, as it does in the normal control, but begins to deteriorate after 60 minutes of hypotension. In the control dog, vasoconstriction of the larger

arteries and arterioles develops and persists throughout the shock syndrome. In the arenal animal, arteriolar vasoconstriction becomes less pronounced so that vasodilatation may be present in these animals during the terminal stage. The over-all blood flow through the capillary bed during shock is inefficient and the return of blood to the venous side of the system is inadequate Transfusion has only a transient mechanical effect on the peripheral circulation, which begins to deteriorate almost immediately after the termination of the transfusion At death, there is marked congestion of the venous side of the vascular bed, a feature which is characteristic of the irreversible phase of hemorrhagic shock.

Shorr Thus, the picture is completely altered so far as the terminal vascular bed is concerned. We have found that excluding the kidney from the circulation profoundly alters the mortality of rats subjected to drum shock (40) Twenty-four hours after drumming, mortality was 29 4 per cent in the controls and 71 per cent in the arenal animals Dr Baez has developed a standardized procedure for hemorrhagic shock in the rat by modification of the Lamson self-infusion device The mortality rate of 14.4 per cent was raised to 69 5 per cent when the kidneys were excluded from the circulation They tolerated less bleeding and they took up more blood than animals with intact kidneys

One can say, then, that the exclusion of the kidney from participation profoundly affects the outcome of hemorrhagic and traumatic shock and that direct visualization of the blood vessels shows that there are profound alterations in the behavior of the terminal vascular bed with blunting or absence of those manifestations which can be regarded as compensatory VEM is absent from the circulation and there is an early deterioration of the liver mechanisms.

Burch Do you think the studies indicate that there is something peculiar to the kidney itself, without altered circulation, or a peculiarity related to a kidney with a reduced blood flow? The same problems might apply to the spleen with and without the splenic artery ligated Alteration in the circulation to the organ might add insult to an already insulted physiology Didn't you ligate the blood vessel and leave the kidney in?

Shorr: That is right

Burch. This could be associated with release of a toxic substance (whatever that means). You leave the spleen in place and ligate the splenic artery.

Shorr· I should say every shock procedure ligates the spleen.

Burch. You mean physiologically?

Shorr. Yes

Fine Did you do any series with nephrectomized animals?

Shorr We have studied it in nephrectomized animals.

Burch· Were the results the same?

Shorr Yes.

Selkurt. In terms of total amount of renal blood flow, I wonder how much blood is needed to perfuse these kidneys in order to bring out VEM, and whether there is some lower level below which you don't get any output of VEM, although the flow may be discernible

Shorr I hope that renal physiologists will tackle that.

Selkurt We have, as a matter of fact Very briefly, I might say that in hemorrhagic shock in a procedure such as yours, done on the dog, only rarely does the renal blood flow stop entirely. This is with direct measurements of renal blood flow During the 50 mm blood pressure period, the blood flow may be as high as 70 or 80 ml per minute per kidney (41). In the final low phase of 30 mm it drops considerably, but only very rarely is there absolutely no flow Dr. Martin Brandfonbrener of our laboratory tried the use of Dibenamine, which does improve the renal blood flow in the hypotensive phase but has no effect on the outcome The dogs die just as readily with or without the drug (42)

Shorr When the drug is given late?

Selkurt When the drug is given during the hypotensive phase The flow may increase quantitatively Percentagewise, it may often double or more Quantitatively, it may go from 35 ml per minute to 50 or so more per kidney, but there is no effect on the outcome In the series done in our laboratory, the survival rate was just about the same in those dogs which received the drug as in those which did not The point I am making is that there is always renal blood flow, and alterations in terms of the volumes that I have recorded have, apparently, no influence

Shorr Then, the crucial point is, how much flow is there in the unanesthetized animal as compared to the anesthetized?

Selkurt These, I might add, are anesthetized animals

Shorr You have no data on the unanesthetized in shock?

Selkurt There are none that I know of.

Shorr Our inference would be, from the continuous appearance of appreciable amounts of VEM throughout this period of drastic hypotension, that renal blood flow is significantly higher in the unanesthetized animal

Fine. Even if it is higher, there is no output of urine, is there?

Shorr That I don't think is relevant

Selkurt: The only way to get such data would be with clearance techniques, and with hypotension I am afraid they would not yield crucial evidence That is why we went to the direct method in the anesthetized animals But I still contend that you have adequate blood perfusing the kidney to wash out VEM, and I don't quite see why it should not appear.

Shorr· I would say there are three things that happen. first of all, through sustained anoxia or hypoxia, the kidney progressively loses the capacity to form VEM, secondly, the amount that appears in the circulation progressively falls and then disappears, thirdly, there is a progressive predominance of the vasodepressor We have found a reduced capacity to form VEM after two hours of that degree of hypotension, so that although there is some blood flow, it is not adequate to keep concentrations high enough to be significant

Moe May I ask what were the survival times in those that died? Did those without the kidney die early in the procedure or late? After a lapse of two or three hours, would the figures be the same for both groups?

Shorr The survival figures were virtually the same at four hours and at twenty-four hours in the rats

Green If you took one group of anesthetized and another group of unanesthetized animals, and adjusted the hypotensive period so that you could get about the same degree of survival, would you find about the same amount of VDM in both groups?

Shorr Yes

Green You may have to set the hypotensive level a bit lower in the unanesthetized dogs

Shorr When the kidney is physiologically excluded by the Lamson technique of lowering the blood pressure rapidly, the unanesthetized animals go into shock

Green Under those conditions, would you get about the same amount of VDM in the anesthetized and the unanesthetized groups of animals? Dr Zweifach at the last meeting, I believe commented that VDM is not found in unanesthetized animals

Zweifach: Dr Green inquired whether the formation of VDM occurs in unanesthetized dogs subjected to hemorrhage The answer is yes The differences between anesthetized and unanesthetized dogs lie in the amount of blood loss and in the degree of hypotension which must be maintained in order to bring about decom-

pensatory phenomena and the formation of vasodepressor principles Dogs subjected to bleeding, without systemic anesthesia, require significantly larger hemorrhages in order to lower the blood pressure to the point where vascular decompensation develops For example, dogs anesthetized with pentobarbital may require about 5 per cent blood loss and a hypotension of 40 to 50 mm Hg for 90 to 120 minutes in order to precipitate circulatory collapse and irreversibility Unanesthetized dogs, on the average, require as much as 5 5 to 6 per cent of blood loss and the rigid maintenance of the blood pressure below 35 mm Hg for two to three hours in order to achieve the same type of circulatory collapse Thus, anesthesia acts as a predisposing factor which facilitates the elaboration of VDM However, anesthetic agents are not a necessary prerequisite to VDM formation Apparently, the anesthetic agent interferes with the capacity of the cardiovascular system to compensate following blood loss, since unanesthetized dogs maintain an adequate circulation through the capillary bed of the omentum at pressures as low as 35 mm Hg

Shorr With the graded hemorrhagic procedure, the unanesthetized animals, which would not ordinarily shift to anaerobic liver metabolism, did so when the kidney was tied off

Zweifach In other words, the statement that VDM appearance is associated with anesthesia is not a correct one?

Green That is what I wanted to bring out

Burton Dr Shorr, may I try to clarify these last experiments? It seems to me that they prove merely that an animal without the kidney function does not get along as well as an animal that has it Surely the experiments don't carry any direct proof that the reason the animal is not so well off without his kidney is that the kidney supplies VEM I mean, might there be something piled up, such as an anuria, which is now not being removed, and makes the situation worse?

Shorr The shocked animal is relatively renal insufficient Whether the kidney is tied off, or whether the blood flow is reduced to such an extent that there is no excretory function, the animal is nephrectomized in either case Therefore, I don't believe piling up of urinary constituents represents the difference What we point out is that the compensatory mechanisms, which we have observed to occur only when VEM is present in the blood, fail to occur in these animals We, therefore, infer that these compensatory mechanisms are referable to the presence of VEM

Engel Ligating the ureter and doing the experiment will answer the question

Shorr. That is right

Engel. Has it been done?

Shorr. No, it has not been done, but that will answer it.

Moe. You don't have any formation of urine?

Shorr. There is none in either case The animal is physiologically nephrectomized

Green: We did a couple of experiments along that line, and could not show that an animal with his ureters ligated was any more susceptible than a normal dog In a cross circulation experiment, reinfusing urine did not make the recipient dog any more susceptible As a result of these observations, I am not sure that the urinary excretory products are of much importance in determining survival in short term experiments (43,44)

Fine We also did experiments with nephrectomized animals They did not show a different response to hemorrhage than animals which were not nephrectomized

Nickerson. Was that a very severe hemorrhage?

Fine Our type was

Shorr The severely hemorrhaged animal is nephrectomized at once, as far as VEM production is concerned

Fine I wonder why you should get a difference in result If the bleeding itself is a nephrectomy, I don't see why ligation of the artery should make much difference

Shorr The difference is in the method of bleeding Inasmuch as the kidney is the only source of VEM, in our graded hemorrhage we obviously have, during the progress of the syndrome, a phase in which the kidney assists the compensatory mechanisms But if the blood pressure is brought down abruptly, as in your experiments, the blood flow is low enough to exclude VEM from appearing in significant amounts That animal resembles one in which the kidneys are ligated

Fine We have some data on the renal flow in these animals, and also on the venous oxygen saturation from the renal vein These are not negligible things, as Dr Selkurt points out There is still a good deal of flow

Shorr It is a matter of how much that flow can contribute to the blood stream in the form of VEM, by actual measurements it is undetectable.

Fine The figure I have on flow from the left renal vein in the normal animal is 230 ml per minute when the blood pressure was

30 mm of Hg In four animals in shock the flow from the left renal vein was 52, 100, 25, and 27 ml per minute respectively The renal venous oxygen saturations were 56, 56, and 36 per cent respectively in the first three. I don't know whether that would be equivalent to shutting off all VEM flow from the kidney, or not.

Fremont-Smith The only way of answering that effectively would be to do an assay of VEM in the blood of such an animal Without that, you are arguing when the crucial question is unknown It could be settled so easily if it is an important item

Moe Presumably we have a relatively narrow range of renal blood flow within which VEM production will be maximum and effective? If there is too much flow, you will have none, and if you have too little, the function will be impaired? If by some magical means, you could insert a transformer into the renal artery so as to jack the pressure up, you would have essentially the same situation as in the arenal animal?

Shorr Yes, and perhaps that is one of the effects of Dibenamine pretreatment

Moe It seems to me that if the Dibenamine abolishes the renal vasoconstriction which occurs early in hemorrhagic shock, then it should be deleterious rather than beneficial

Shorr It is not a question, I think, of abolishing all constriction, because the blood vessels still constrict upon the reduced blood volume In other words, factors other than those that are depressed by Dibenamine operate to produce some degree of vasoconstriction, which is evident by direct inspection, and that appears to be sufficient to produce enough renal hypoxia for the release of VEM, but it doesn't permit the extreme and exaggerated vasoconstriction which occurs in drastic hypotension in the normal animal

Moe In other words, you expect to keep the renal flow in the intermediate range?

Shorr Yes, in the intermediate range

Nickerson Dr Shorr, I am not clear as to why VEM is not released during a drastic reduction in renal blood flow It does not seem to me reasonable that the flow is simply inadequate to wash it out Even at 50 ml a minute, there is quite a lot of solvent going through the kidneys Also, a considerable amount of VEM should be produced If you suddenly place kidney tissue under anaerobic conditions by putting it in a vessel under nitrogen, or by completely occluding the renal artery, VEM production continues for a con-

siderable period of time Why does not this VEM appear in the circulation?

Shorr I would say this wherever there is any blood flow through the kidney, VEM is going to come out, but the extent to which it can attain a significant concentration in the blood stream is the crux of the matter Apparently in the drastic hypotensive phase so little is released that we cannot detect it by our methods We suspect, from experiments in which drastic hypotension has been maintained, and then assays made on the kidney cortex showing little or no VEM to be present, that an anaerobic deterioration of the mechanisms responsible for its production occurs

Nickerson· Why is that not true of the Taquini procedure, which produces very high concentrations of VEM?

Shorr If the hypoxia is maintained as long as in the Taquini procedure, only the depressor factor is formed, there is no VEM effect (45,46).

Nickerson We are talking about the first hour or so after Dr Fine's sudden reduction of pressure It seems to me that there is one possible explanation of this apparent dilemma

Shorr. Do you mean subcortical vessel medullary flow?

Nickerson Yes Are these small amounts of blood actually passing through the area of VEM formation?

Shorr That is a very good point, because it may very well be that these small amounts are actually not flowing through the cortex where the VEM is formed

Moe Have you done differential slice studies, Dr Shorr?

Shorr. As far as we are able to separate medulla and cortex, the VEM production is in the cortex

Nickerson I suspect that Dr Selkurt would like to say something on this point

Selkurt I should like to be able to distinguish between the medullary and cortex flow under these conditions and decide whether the situation is as you stated Unfortunately, I cannot There is a possibility that it might exist Many of the examinations of flow, in terms of clearance techniques, and so on, have indicated that the shunt does not operate, certainly in dogs However, I would be less inclined to take that adamant point of view in terms of the circulation through the rabbit kidney, in which the possibility exists In view of the facts a medullary shunt, alone, is probably not going to be the answer in the dog However, until we examine the point under specific conditions we cannot say that it could not be a possibility

Green Does bleeding the rabbit with the Lamson bottle cause a greater reduction in renal flow?

Shorr If the blood pressure were lowered 30 mm abruptly with the Lamson bottle, what would happen?

Selkurt I personally never use that technique We bleed down to 50 mm, rather rapidly initially, but under those circumstances it never gives a renal shutdown The flow is very adequate at first, and sometimes not less than half of the control flow, then we wait and bleed later to 30 mm, and that does reduce the flow As I stated earlier, very rarely is the flow shut off Sometimes, later in the period, it may be

Shorr Perhaps you can tell us the next time what happens if you follow Dr Fine's procedure of abruptly reducing the blood pressure to 30 mm

Fine The figures I gave you were for total venous outflow per minute from a catheter tied into the left renal vein

Selkurt That would be the same technique we have used in our studies

Lawrason. Dr Shorr, I am a little bit bothered about the analogy being made in these four-hour and 24-hour survival studies between the dog in shock and the nephrectomized dog It seems to me that there may be many other factors playing a role in survival, and to say there is a close similarity between the two may not be wise

Shorr For the rat subjected to drum shock, the actual mortality figures at four hours were only three per cent different from those at 24 hours I would offer those as suggestive that not too many other complicating factors introduced by the removal of the kidney would be operative

Lawrason Have you tied the renal artery or vein with controlled releasing before and after drumming?

Shorr No, we have not, and I think perhaps the point you raised could be answered if we tied the ligatures in our control series, after the drumming was finished It is a very good suggestion, and I think that by tying the kidney afterwards we would have both types of animals nephrectomized for the 24-hour period However, I should like to emphasize the very small difference between the mortality at 24 hours and at four

Fine What does the peritoneal cavity show in these rats? Did you look to see if there is any fluid in the peritoneal cavity?

Baez Immediately after death, the arenal rat's liver consistently was found to be hard, engorged, and dark A small amount of fluid

siderable period of time Why does not this VEM appear in the circulation?

Shorr: I would say this: wherever there is any blood flow through the kidney, VEM is going to come out, but the extent to which it can attain a significant concentration in the blood stream is the crux of the matter. Apparently in the drastic hypotensive phase so little is released that we cannot detect it by our methods We suspect, from experiments in which drastic hypotension has been maintained, and then assays made on the kidney cortex showing little or no VEM to be present, that an anaerobic deterioration of the mechanisms responsible for its production occurs

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the same result would apply to nerve stimulation, but I think it would

Shorr. I think that is a suggestion worth following.

I should like to outline our findings in rats which are resistant to drum shock (47) We have used the method of Noble (48) for producing resistance, and have found a number of changes in the ferritin inactivating mechanism, which may be related, by inference only, to the acquired resistance to shock.

Livers of resistant rats were able to inactivate ferritin more rapidly than livers of control animals When subjected to prior anaerobiosis and then returned to oxygen the liver inactivation mechanism of the resistant rats was almost unchanged, whereas the livers of control rats inactivated much less ferritin than before. I believe this may represent a quantitative increase in the inactivation mechanism of the resistant rat, rather than a qualitative change.

Moe Doesn't that look more like a qualitative difference, if the enzyme system is resistant to anoxia here, and is not in the normal liver?

Shorr There are two possibilities that the enzyme system is altered or that there is now twice as much, let us say, of the same enzyme system I would be more inclined to think it is a quantitative rather than a qualitative change in enzymes.

Moe This sort of thing sounds familiar to us from Syracuse, because Jay Tepperman often speaks of growing new enzymes

Shorr Without anaerobic pretreatment the resistant rat liver inactivated faster than the control liver, rather suggesting a quantitative change The oxygen consumption of both resistant and control rat livers was profoundly depressed by prior incubation in nitrogen, although, as I said before, the inactivation mechanism of the resistant liver was largely unharmed Thus the ability to inactivate ferritin appears totally unrelated to the over-all energy metabolism of the liver cell Control rats were subjected to 700 revolutions of the drum and resistant rats to 1000 There were high titers of VDM in blood and liver wash in the control rats, and minimal amounts in the resistant The liver VDM inactivation system was profoundly impaired in the controls, and virtually intact in the resistant rats The mesenteric capillary bed was hyporeactive in the controls, and normal or even slightly hyperreactive in the resistant rats Thus, a profound change, probably enzymatic in nature, occurred in the resistance to anoxia of the VDM inactivation system of the liver in the trained rats

was present in the peritoneal cavity, but no more than in a shocked normal rat.

Fine: You say there is no difference between the normal and the arenal?

Baez: The normal animal in the irreversible stage of shock presents the same picture. The intestinal wall is cyanotic and congested The liver is congested and hard

Fine I may sound academically fussy about it, but I bring it up because I think the state of uremia has something to do with the permeability of the intestinal wall to bacteria. When we tried to get transmural migration of intestinal bacteria by irrigating the peritoneal cavity, we got nowhere until we made the dog uremic, then we got a peritonitis and could show that isotope-labelled bacteria in the gut moved across the wall into the peritoneal cavity. I don't know whether that is actually the case in your rats A difference in your two series may be due to such a factor I doubt whether culture techniques would be sufficiently sensitive to demonstrate such a phenomenon

Shorr I think Dr. Lawson's suggestion that we tie the kidneys of the control animals after drumming might be a good one to introduce the same arenal element into the recovery picture We will do that

Nickerson The difficult problem would be to decide when to tie the kidneys This is presumably an effect which comes on gradually and disappears gradually over a fairly extended period of time

Shorr: Perhaps at the end of four hours.

Nickerson: I do not know.

INFLUENCE OF SYMPATHETIC BLOCKADE UPON DEVELOPMENT OF SHOCK

Moe: I wonder whether you have done any experiments with the dihydrogenated ergot alkaloids in this problem?

Shorr. No, we have not

Moe The reason I ask is that in a few acute experiments in which we studied the effect of epinephrine and nor-epinephrine upon renal blood flow in normal animals, Dibenamine did not greatly reduce the renal vasoconstrictor effect if given in doses which caused reversal of the blood pressure response to epinephrine, whereas dihydroergocornine (DHO-180), in doses which potentiated the pressor effect of epinephrine, almost completely eliminated the renal vasoconstrictor effect I don't know whether

Shorr. Yes, these were all arenal Some were given no Dibenzyline and others received 20 μg per 100 gm. body weight before drumming

Moe: Therefore, this certainly suggests that the action of Dibenzyline is not exerted on the kidney.

Shorr. Not that it has no effect on the kidney, but that its crucial influence is not due to the participation of the kidney For example, in the Noble-Collip drum trauma with resistant rats, renal ligation has no influence on the 24-hour survival. Just as many survive 24 hours without the kidneys as with, provided the animals are trained and have developed an enhanced liver inactivation mechanism.

Moe The second kidney is ligated shortly before the drumming?

Shorr. That's right

Moe: And the Dibenzyline is given an hour before?

Shorr. Sixty or ninety minutes

Moe. That suggests to me that one need not worry about what Dibenamine, Dibenzyline, DHO, or anything else, does to renal blood flow, you are not protecting the animals because you are maintaining renal blood flow

Shorr. It may be that the difference between 81 and 100 per cent survival is represented by the kidney's contribution.

Burch: Of course, it is not possible to be sure that it has anything to do with the blood flow of either

Shorr. You mean in the kidney?

Moe. Or the liver

Shorr I think we are able to show that it is significant.

Nickerson There is suggestive evidence, however, that the inhibition of sympathetic activity is involved The studies with Dibenamine are very similar to the old experiments of Freeman, *et al* (49), in which they found surgical sympathectomy to provide excellent protection against shock I believe the suggestion that Dibenamine is acting by inhibiting sympathetic activity is probably valid, although we cannot say where it is acting or at just what stage of the shock process

Moe Did you ever study the effects on any branch of the splanchnic circulation?

Nickerson: Not as an isolated circulation

Shorr In a little while I am going to ask Dr Zweifach to tell you about the changes he observed in the splanchnic circulation

Adrenalectomized, salt-maintained animals are very susceptible to any kind of shock For example, one series was drummed 600 times with none surviving. Prior treatment with 20 μg Dibenzyline

Rats which have been made resistant to drum trauma are also more resistant to hemorrhagic shock. When subjected to hypotension of exactly comparable degree and duration, mortality was 60 per cent in the controls as compared with 20 per cent in the resistant rats. The control rats had VDM in plasma and liver wash with profound deterioration to total loss of VDM inactivating capacity. Although blood from the resistant rats also contained VDM, the livers retained their normal capacity to inactivate VDM on aerobic *in vitro* incubation. We infer, from these experiments, that the preservation of the hepatic inactivation system may be one of the factors contributing to the increased resistance to shock.

Doctors Zweifach and Baez carried out experiments in hemorrhagic and traumatic shock which were designed to investigate the relationship between the protection afforded by Dibenzyline pretreatment and these humoral factors. Dr. Baez, how much Dibenamine was given to the rats?

Baez. Twenty micrograms of Dibenzyline (SKF 688-A), a derivative of Dibenamine.

Shorr. With this dose there was virtually no difference from the normal controls in the maximal blood loss required to maintain the hypotension. The uptake of blood was less and there was a higher percentage of survival than in the controls.

Moe. That was twenty micrograms?

Shorr. Per 100 gm. of body weight.

Nickerson. It is important to distinguish between Dibenamine and Dibenzyline (SKF 688-A). The latter is eight or ten times more potent.

Zweifach. The dosages referred to above are all with reference to the drug called "Dibenzyline." Similar effects were also obtained with the original drug, Dibenamine, except for the fact that higher doses had to be administered in order to achieve a protective action.

Shorr. The mortality was 80 per cent in the untreated controls. It was 12.5 and 6.6 per cent respectively with 5 µg. and 20 µg. of Dibenzyline given 60 to 90 minutes before bleeding. Following 750 rotations in the drum 46 per cent of the untreated rats survived, 100 per cent survived when treated with 5 µg. and 93 per cent with 20 µg. These doses were given 60 to 90 minutes prior to drumming. Thus Dibenzyline protects against drum shock.

In arenal rats, too, protection is exerted by Dibenzyline, 81.8 per cent survived as compared with 20 per cent in untreated arenal rats.

Moe. These are all arenal?

tive throughout the syndrome There was no appreciable loss of arteriolar tone. An extraordinary observation was the maintenance of an effective capillary blood flow at blood pressure levels as low as 30 mm Hg. The protective action of Dibenzyline would appear to reside in this particular observation In other words, despite the lowering of the blood pressure to levels as low as 30 mm. Hg, the circulation through the terminal vascular bed persisted sufficiently to maintain tissue integrity. Thus, Dibenzyline would prevent the development of tissue hypoxia, despite drastic levels of hypotension. Shock, in so far as the terminal vascular bed was concerned, did not develop in the Dibenzyline-treated animal

Nickerson. Am I correct in assuming, from these experiments, that there was considerably less arteriolar constriction in the Dibenzyline-treated animals?

Zweifach Vasoconstriction was less pronounced in the arteries and arterioles of the omentum in the Dibenzyline-treated animal.

Fine What was the degree of blood volume deficiency?

Zweifach Blood loss is indicated in terms of per cent of body weight. We do not have data on the per cent of the total blood volume which was removed in these animals At the end of the period of hypotension, all of the blood which had been removed was infused back into the animal About 20 to 30 ml of this total were removed for rat assay studies.

Fine What was the total duration of the lowest pressure?

Zweifach An attempt was made during the experiment to maintain the blood pressure below 40 mm Hg for almost four hours After the fourth bleeding, the pressure fell to 30 to 35 mm Hg However, there was a tendency for the blood pressure to rise spontaneously In order to achieve a suitable hypotensive level, it was necessary to withdraw successively small amounts of blood This final stable range, during which no further bleedings were required to sustain the hypotension, persisted for approximately 2½ hours On the basis of previous experiments, anesthetized animals subjected to this degree of drastic hypotension invariably become completely refractory to transfusion therapy In my opinion, the Dibenzyline (SKF 688-A)-treated dogs were punished more drastically than control animals Despite this, the treated animals could be recovered by transfusion It is interesting that animals treated with Dibenzyline can be recovered by replacement of as little as 50 per cent of the total blood withdrawn during hemorrhage One is struck by the similarity between the circulation in sympathectomized dogs and in Dibenzyline-treated animals follow-

per 100 gm body weight resulted in 100 per cent survival. To me, this is most extraordinary for such rats will have 100 per cent mortality with many fewer drummings than 600 The adrenalectomized, salt-maintained rat, without having anything done to it, has a neutral plasma, a neutral liver wash, will not produce ferritin under aerobic incubation, and has a fair but not entirely normal, inactivation capacity. After 600 rotations in the drum with and without Dibenzyline, there is a striking difference: without Dibenzyline, mortality is 100 per cent with a large amount of ferritin in plasma and in the liver wash, and the liver actually produces vasoactive ferritin on incubation in oxygen, having completely lost its inactivating capacity. With Dibenzyline protection, however, the plasma and liver wash remain neutral, the liver does not produce ferritin in oxygen and the inactivating mechanism is unchanged Hence, these experiments support our hypothesis that this particular system is related to the recovery from hemorrhagic and traumatic shock

Dr Zweifach, would you describe the effect of hemorrhage on the circulation, as modified by Dibenzyline?

Zweifach The vascular changes observed in control, untreated dogs were listed in Figure 20 These were characterized by the progressive deterioration of vascular compensatory mechanisms and the onset of hyporeactivity. The response of the terminal blood vessels to epinephrine became depressed, spontaneous vasomotion disappeared, and capillary circulation was extremely sluggish Following transfusion, the arterioles became atonic and distended, the capillary circulation remained slowed despite blood pressures as high as 100 mm Hg, and progressive pooling of blood on the venous side of the bed developed During this phase of the syndrome, blood samples continued to show vasodepressor activity, which persisted despite perfusion

In contrast to this was the situation in a dog treated with Dibenzyline (SKF 688-A) 14 hours earlier An attempt was made to carry out identical states of hypotension in the two experiments The Dibenzyline-treated animal remained reversible The hyperreactive response to epinephrine, although somewhat blunted, was quite evident in these animals, an increase of five hundred fold from the initial threshold value being obtained It was interesting that Dibenzyline (SKF 688-A), which blocked the pressor action of intravenous epinephrine at this point, had no blocking effect on the direct response of the terminal vascular bed to topical epinephrine Vasomotion became augmented and remained effec-

animals In the present experiments, an attempt was made to maintain comparable levels of hypotension in the two sets of animals for identical periods of time This could be achieved only by graded withdrawal of blood in the manner indicated, otherwise a direct comparison between the two could not be effectively made.

Knisely Do you think that the drug acts by a relaxing action on the terminal arterioles? You see, the key point is that to whatever degree the Poiseuille equation applies, the volumetric flow is a function of the fourth power of the radius, so a very small relaxation will permit a great deal of flow

Zweifach That may very well represent the locus of the protective action of Dibenzyline in these experiments

Stead Is there any difference in blood volume?

Zweifach. Blood volume studies have not been carried out

Burch· What happens to the arterial blood pressure in the Dibenzylene-treated dogs?

Zweifach On the average, the control blood pressure levels of the Dibenzyline-treated animal were the same as those of untreated dogs

Burch The arterial blood pressure did not drop? Did you treat them a few days in advance?

Zweifach 688-A was administered at different times, varying from 2 to 18 hours, before the experimental hemorrhage Except for a transient fall at the time of administration of the drug, the blood pressure level was maintained in essentially the normal range. Even in untreated dogs, the control levels of blood pressure varied from animal to animal by as much as 40 mm Hg

Fine Does anybody know what Dibenzyline does to the normal blood volume?

Nickerson I do not know No one, so far as I know, has studied that problem

While I have the floor, I should like to say a few words about the pharmacology of Dibenamine and its congeners The terminal vascular bed was very sensitive to topically-applied epinephrine in the animals treated with Dibenamine When I first saw these figures made by the Cornell group, I simply did not believe that Dibenamine would not block the responses of the terminal vascular bed to epinephrine Consequently, in cooperation with Dr Bohr, we set out to study this problem, using the rat meso-appendix technique We found that Dibenzyline effectively blocks the response of the terminal vascular bed to epinephrine It will reduce the responsiveness from a level of 1 3 to 1 6 million in the control,

ing hemorrhage. Both show a remarkably effective blood flow through the terminal vascular bed at drastic levels of hypotension. Actually, levels of hypotension as low as this cannot be maintained in a normal untreated dog under anesthesia for more than 10 to 15 minutes without precipitating circulatory collapse.

Selkurt. Did you normally bleed more or less in these Dibenzylineized dogs?

Zweifach: The Dibenzyline-treated dog must be bled carefully. When such animals are subjected to drastic or acute massive hemorrhage, they collapse. However, by progressive, careful removal of blood, one can finally achieve a total hemorrhage which is well within the range of that obtained in control experiments.

Burton: Didn't Freeman (49) find it took much less bleeding?

Zweifach In Freeman's experiments on sympathectomized dogs, an acute withdrawal of blood was used to produce hypotension rapidly. In such experiments, the total blood loss which was tolerated by the sympathectomized dog was significantly less than that of control animals We have found that the sympathectomized dog, like the Dibenzyline-treated animal, can tolerate fairly extensive bleedings provided the withdrawal of blood is carried out progressively over a protracted period of time

Burton Don't you find that it takes less bleeding to get down to 30 mm pressure than it does using an animal without the drug

Zweifach· Yes However, this pressure is not maintained, and unless successive bleedings are carried out the pressure will return to levels as high as 70 to 80 mm Hg At the end of the experiment, the total blood loss which is tolerated by both groups of animals is approximately the same The tolerance to blood loss in the different groups of animals under discussion depends on the experimental procedure which is being used There is no doubt that both the sympathectomized and Dibenzyline-treated animals are unable to compensate for the acute withdrawal of large amounts of blood For example, the acute withdrawal of about 3 5 per cent of blood in terms of body weight will usually precipitate circulatory collapse and death in either The same bleeding carried out in a normal dog results only in the development of a state of hypotension Under these experimental conditions, then, it can be stated that the experimentally-treated animals withstand less blood loss than the normal controls However, when one withdraws blood carefully, using both the changes in the omental circulation and the blood pressure as a guide, it is possible to remove an amount of blood by graded procedures which, in the end, is equal to that removed from control

Dibenzyline. The latter received 20 μg per 100 gm of body weight 90 to 120 minutes prior to the first bleeding The bleeding was carried out by a method previously described by our laboratory (50), which consists essentially in the use of a Lamson type self-infusing device adjusted for small animals. A state of moderate hypotension with blood pressure of 70, and then 50 mm Hg was maintained for two hours, and for a subsequent two-hour period the pressure was kept at the more drastic hypotensive level of 40 mm Hg. At the end of this time the blood remaining in the reservoir was slowly infused. Records were kept of the "bleeding-out" volume, the time at which maximal bleeding was attained, and the duration of maximal bleeding before spontaneous uptake occurred Behavior of the terminal vascular bed during the evolution of the shock syndrome was followed by direct microscopic observation of the meso-appendix

Although the hypotension achieved was equivalent in the control and treated animals, about 15 minutes after attaining the maximal bleeding volume, the control animal took up blood from the reservoir, whereas the Dibenzyline-treated animal showed almost no spontaneous uptake Following transfusion, there was only a temporary restoration of normal blood pressure in the control animal, which died eight hours later. In the Dibenzyline-treated animal, on the other hand, restoration of normal blood pressure persisted and the rat was alive and in excellent condition 24 hours afterwards.

The hemodynamic effects of Dibenzyline during the development of shock in the rat duplicate essentially what we and Dr Zweifach have reported in the dog, namely, considerable blunting of vasoconstriction of the small arteries and arterioles Local homeostatic mechanisms in the capillary bed, however, remain unimpaired as evidenced by a progressively enhanced vasomotion and potentiation of the vascular response to topically applied epinephrine.

A point which perhaps has a bearing on Dr Nickerson's remarks is diagrammatically represented on the right side of Figure 21 Dibenzyline was given intravenously 90 to 120 minutes prior to the start of the hemorrhagic procedure In these animals we invariably found that the basal reactivity of the terminal vascular bed of the mesentery to topically applied epinephrine was depressed (1 100,000 to 1 500,000) as compared with normal rats which have a basal reactivity of 1 2 to 1 6 million As you will note, however, in the treated animal the reactivity to epinephrine progressively increased and at approximately one hour reached a level comparable to that in a normal animal At this time the bleeding equalled

to a point where it will not respond, or will just respond, to a dilution of 1:1000. The trick is that testing must be done at just the proper time interval after administration of the blocking agent. Dibenamine or Dibenzyline will block, but their metabolic products apparently act as sensitizing agents. A dose of Dibenzyline which will block the responses of arterioles for 24 hours or longer will reduce the sensitivity of the terminal vascular bed during the first hour or two after administration However, 8 to 24 hours after administration, the terminal vascular bed is markedly sensitized. Sensitization can be produced without prior desensitization by giving Dibenzylamine or 2-dibenzylaminoethanol, which are degradation products of Dibenamine.

Shorr. Dr. Baez has followed up your work, and I should like him to tell us of his experiments.

Baez. In Figure 21 hemorrhagic shock is illustrated on the left in the normal rat, and on the right in the rat pretreated with

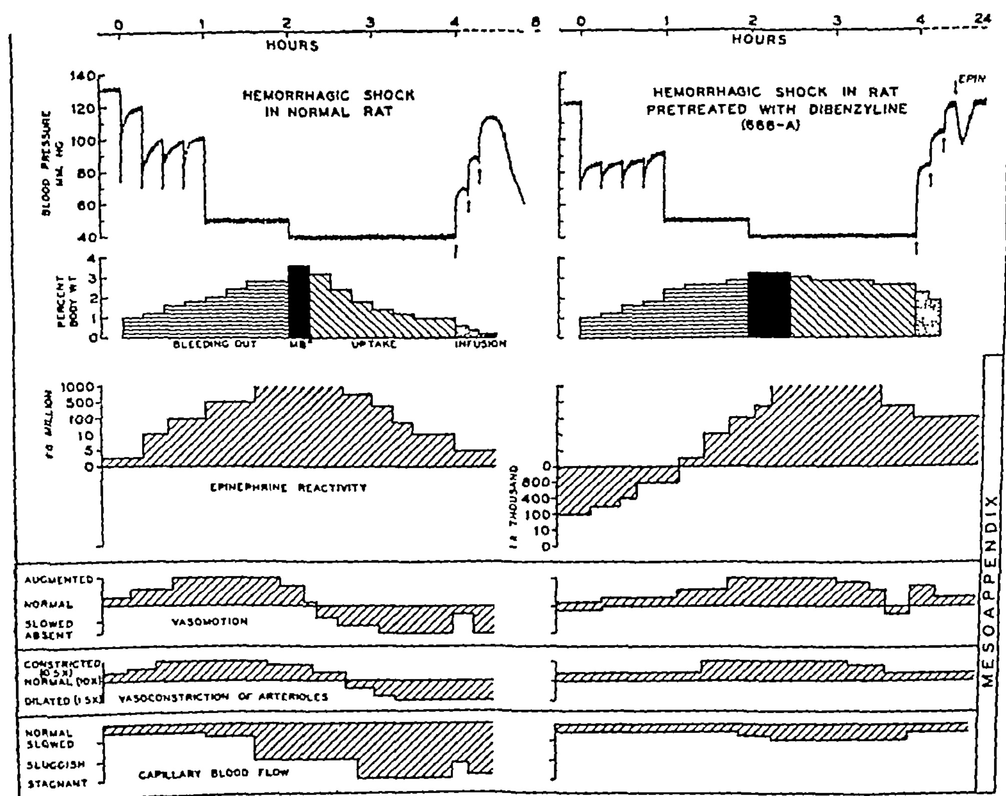


FIGURE 21 Peripheral vascular responses to standardized hemorrhagic shock in the rat as modified by pre-treatment with Dibenzyline (20 micrograms/100 gm body weight, intravenously, 60 to 120 min prior to start of bleeding) In this series of experiments the mortality was 80% in the control rats and 6 6% in the rats pretreated with Dibenzyline and subjected to equivalent degree and duration of hemorrhagic hypotension by means of modified Lamson self-infusion technique

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1.8 to 2.0 per cent of the body weight. In other respects, 90 to 120 minutes after the administration of Dibenzyline, the meso-appendix circulation appeared essentially normal. Spontaneous vasomotion in the normal animal is exaggerated soon after the onset of hemorrhage. In the Dibenzyline-treated animal, however, the enhancement of spontaneous vasomotion started later, and was maintained for a longer period of time. In the normal rat spontaneous vasomotion becomes impaired at about 140 to 150 minutes whereas it persisted in effective functional state in the Dibenzyline-treated rat. The venules and collecting veins present a narrowing and occasional varicose constriction which, I think Dr Zweifach will agree, is what occurs in the Dibenzyline-treated dog during hemorrhage. The Dibenzyline-treated rat remains in a compensatory state at the end of the experiment and most of the available circulating blood is on the arterial side of the vascular tree and not on the venous side as it is in control animals (50).

Zweifach: It is apparent that hyperreactivity will develop in the terminal vascular bed, despite adequate adrenergic blockade in terms of the blood pressure response to epinephrine.

Nickerson: Yes, and apparently, from our experiments, a part of that hyperreactivity may actually be due to materials formed from Dibenamine in the body. Hyperreactivity can occur several hours after the administration of Dibenzyline even though the animals are not subjected to shock or other procedures.

Zweifach: It is unlikely that the protective action of Dibenamine or Dibenzyline is related to the elaboration of degradation products of these compounds. Irrespective of whether the drug is given 90 minutes, 2 hours, or 24 hours prior to the shock experiment, the treated animals are protected against the deleterious effects of shock as long as a dose sufficient to produce adrenergic blockade is administered. Whatever the locus of action of the drug, the basic difference between protected and unprotected rats is the persistence of an adequate circulation through the capillary bed at extremely low levels of blood pressure.

Nickerson: I would interpret that as another bit of evidence indicating that the protective effect of these drugs is at the level of the arterioles rather than on the terminal vascular bed.

Engel. I take it, then, that in the situation you are dealing with, liver metabolism is reasonably normal as measured by other metabolic parameters, and that this is being protected. In some of the early experiments Wilhelmi (51) did (actually, they were the last ones by the Yale Shock Group), he had some data indicating how

procedures done previously to the circulation of the liver would improve the liver's capacity to resist anoxia later Using the technique of hepatic artery clamp, he studied the effects on subsequent metabolic behavior of the anoxic liver of the introduction of certain specific substrates, i.e , glucose, amino acids, fats, and so forth, into the gastrointestinal tract some time before the operation It was found that all the agents which were put into the stomach by stomach tube afforded protection, with the exception of corn oil, as I remember The logical thing, at that point, was to do a control in which saline was put into the gut, there being no question of a specific metabolite then It was found that saline put into the gut two hours before the liver was made anoxic protected it equally as well as did any of the other substances tested It was decided that this was an effect on the circulation, which conferred on the liver an ability to resist hypoxia longer than it would without something being introduced into the gastrointestinal tract beforehand

Zweifach Both Dr Selkurt and Dr Fine have brought out an important point in their experiments that should be emphasized, namely, that the administration of Dibenzyline after a state of shock has developed has no protective action The drug must be administered prior to the induction of shock in order to be effective

Engel· In regard to the resistant animals, as I understand it, you are dealing with an entirely different situation You should be able to tell us what the significance of these changes is I take it from your data that the liver, in this case, is actually anoxic, but that you do have a sparing of the VDM inactivating mechanism in spite of the hepatic anoxia. Have you studied any of the other metabolic changes? That is, do these animals have elevations in plasma amino acids, or any of the other changes usually seen with shock?

Shorr: We had hoped you would do that

Cotzias Have surgeons tried to give any of these agents before an operation?

Shorr Ganglionic blocking agents have been used at Memorial Hospital, in New York, and elsewhere to induce hypotension prior to drastic operations in which tremendous loss of blood is anticipated The postoperative picture, particularly the response to transfusion, is profoundly ameliorated They emphasize that the patient must be kept nearly flat

Fine Yes Learmonth and others have been doing it with hexamethonium It produces a severely ischemic field in which

to work. Apparently, since he is a very competent man, he would not be using it if it were deleterious, although it takes a lot of courage

Stead. I should like to point out that vascular relaxation is a part of the natural history of hemorrhagic shock When a dog is first bled, it is difficult to keep its pressure constant at a level of 40 mm. Hg. Further removal of blood results in death, and slight hemodilution results in a rise in pressure The dog is sensitive to gain or loss of blood. After several hours, it becomes easier and easier to keep the dog hypotensive Decreases in blood volume no longer cause death, and increases are not associated with a marked rise in pressure Again, I should like to raise the question of the blood volume and Dibenzyline effect. If a dog in perfect health is exsanguinated immediately, it dies without any benefit from hemodilution If the animal is exsanguinated to the point at which its peripheral circulation nearly stops, it will again hemodilute very slowly On the other hand, if the blood pressure is lowered and, at the same time, the blood flow maintained, that is a perfect situation in which hemodilution is going on at a maximum rate I wonder whether what happens, in part, with the Dibenzyline might not be the same thing that happens in the natural course of repetitive bleedings The lowering of the pressure occurs with less vasoconstriction and therefore better blood flow In the Dibenzyline experiments, I should think it would be important to determine not only the amount of blood taken out but also the amount of blood left in the body The amount of blood left in may be greater than would be expected from the amount taken out

Shorr However, Dibenzyline protects against drum shock, in which the blood volume phenomenon may be less important than in hemorrhagic shock

Fine Are there any data on what happens to the blood volume in the drum-shock rat?

Shorr. I do not think we have any, yet

Fine Wouldn't they be pertinent in interpreting the Dibenzyline effects?

Shorr. They certainly would

Green Have you attempted to see whether you can produce resistance in a normal liver slice by a short period of treatment such as the addition of Dibenzyline, VEM or an enzyme?

Baez. Although I do not think we have a sufficient number of experiments to draw definite conclusions, I may mention a few experiments we have done We should like you to regard these

results as preliminary until they can be expanded. We have found that when liver slices are incubated with Dibenzyline *in vitro*, the ferritin inactivation system seems to withstand subsequent hypoxia better than do liver slices incubated similarly but without Dibenzyline. Also when liver slices, obtained from a rat which had received 20 µg. of Dibenzyline per 100 gm. body weight 90 to 120 minutes before sacrifice, were incubated in Ringer-phosphate solution in an atmosphere of 20% oxygen for 2 hours, no VDM could be demonstrated in the supernatant. On the other hand, there is VDM present in the supernatant of liver slices obtained from control rats and similarly exposed to hypoxia.

Nickerson. Several years ago, we sent several adrenergically active and inactive Dibenamine congeners to Dr. Guzman Barron, who studied their effects on choline oxidase. He found they were all potent inhibitors of this sulfhydryl enzyme, but he found no difference between the members of this group with adrenergic blocking activity and the inactive compounds (52). We know, from our own investigations, that Dibenamine does rapidly link with sulfhydryl groups.

Shorr: That should be looked into.

Nickerson: It should be determined whether this liver effect is related to adrenergic blockade per se, or is, as it very well could be, an effect on the enzyme system involved in the reduction of ferritin.

Shorr: Even if it should be a nonspecific effect, unrelated to the adrenergic blocking effect of Dibenzyline, it would still be of considerable interest in relation to ferritin metabolism in the liver in shock.

Nickerson: It would, and it would then be necessary to see if compounds which do not block the effects of epinephrine, but can alter this liver system, would be protective in shock.

Moe: Has Dibenamine alcohol been tried in shock?

Nickerson: I do not think it has, and it would not be quite appropriate, because the chlorine is necessary for the reaction with sulfhydryl. However, the benzyl group can be changed in many ways to obtain compounds with high chemical reactivity but no adrenergic blocking action.

Shorr: Do you think you could send us some compounds?

Nickerson: I could.

Baez: After the intravenous administration of Dibenzyline (SKF 688-A) the basal reactivity to topical epinephrine of the rat meso-appendix capillary bed becomes depressed. With as little as

1 μg of Dibenzyline per 100 Gm of body weight a decrease of reactivity is noted The extent and duration of the depression seems to be related to the dose given Thus with 20 μg of Dibenzyline per 100 gm of body weight the reactivity of the terminal vessels to epinephrine goes down to less than 1 100,000 and requires five to six hours to come back to threshold levels. The depression of the basal reactivity following the injection of Dibenzyline does not seem to be related to ferritin (VDM) mechanisms since it could not be prevented with the specific antiferritin serum I should like to point out again that in spite of the depth of the depression of the reactivity of the terminal vasculature, it is rapidly restored and becomes hyperreactive under the stress of bleeding equivalent to about 1 5 to 2 per cent of the body weight

Nickerson. We did this same sort of thing in a little different manner We took animals shortly after the administration of Dibenzyline, determined the level to which the sensitivity was depressed, and then administered a previously assayed VEM sample The VEM caused essentially the same magnitude of increase in these animals as in control animals, although starting at a lower level. The only place where our data differ from yours is in the length of time for the sensitivity to return to normal or above I do not recall any experiment in which our animals were not normally reactive, or hyperreactive, eight hours after the Dibenzyline administration, and we have employed doses larger than those which you used

Zweifach Did you try nor-epinephrine as the topical vasoconstrictor for measuring the reactivity of the terminal vascular bed?

Baez With nor-epinephrine, a larger concentration is needed

Zweifach. Is this in terms of blockade by Dibenzyline?

Baez: In terms of reactivity of the terminal vascular bed to topical application of nor-epinephrine

Shorr We have confirmed Dr Nickerson's original observation that these amounts of Dibenzyline depress the reactivity of the terminal vascular bed for varying periods of time The duration of the depression depends upon the dosage Within an hour after the first bleeding, when blood equivalent to 1 5 to 2 per cent of the body weight has been withdrawn, the mesenteric bed is free of the depression of reactivity due to the Dibenzyline and the reactivity mounts to the heights that are seen in the normal animal I do not know the mechanism of this; Dr Nickerson may be able to explain it.

Nickerson: Frankly, neither do I

Moe Could it merely mean that the Dibenzyline in no way interferes with the effect of VEM on vascular reactivity?

Shorr That is correct

Nickerson Dr Moe's point agrees with our observations In our experience, the amount of increased sensitivity induced with a standard amount of VEM is about the same in control animals and in Dibenzyline-treated animals, although in the latter it starts at a lower point and the absolute level of sensitivity reached is not quite as great

Shorr I think that is an indication of the magnitude of VEM production under the hypoxia of shock

METABOLIC ASPECTS OF FERRITIN (VDM) AND OF ANTIFERRITIN

Engel May I ask a question about reduced ferritin from a metabolic standpoint? Have you done any studies at all on the effects of sulfhydryl ferritin in reduced form on enzymatic reactions in the liver, or anywhere else? I am thinking again of your resistance experiment Is ferritin itself responsible for any of the metabolic changes in the liver? Is it an inhibitor of any of the enzyme systems? I am thinking of the observations of Greig and De Turk (53,54), who showed that there was an amino acid oxidase inhibitor which was formed in the liver and released during shock

Shorr We have studied several enzyme reactions as affected by ferritin These include phosphoglucomutase which converts glucose-1-phosphate into glucose-6-phosphate, and the glycolytic system which catalyzes the conversion of glycogen to lactic acid With both of these systems ferritin was found to act as an inhibitor No inhibition, however, was involved when apoferritin was used Inorganic iron salts are also inhibitory, hence one cannot exclude the possibility that the ferritin inhibition is due also to the action of its iron There is increasing evidence that in its action as a vasodepressor, ferritin not only must be in the sulfhydryl form but that its iron is also involved We can, for example, obtain a vasodepressor effect by the injection of iron into the blood stream This action, however, can be virtually abolished by the prior administration of antiferritin serum, suggesting that the iron operates not per se but after becoming attached to ferritin This would also mean that the vasodepressor effects achieved with apoferritin would require that apoferritin incorporate iron following its injection into the blood stream

Engel: What are the effects of other substances which influence SH groups, such as ascorbic acid ion, and so on?

Shorr Ascorbic acid, as well as reduced glutathione and cysteine, will transform oxidized ferritin to the sulfhydryl form

Engel. It is tough.

Burton Ascorbic acid does have a very marked effect on the resistance to cold, as shown by Dugal *

Nickerson: But that effect is in the wrong direction to fit in with this particular explanation.

Burton I have not thought it out, I am just reporting this.

Nickerson Dr Shorr, before we leave the ferritin story may I raise a question which, I believe, was raised last year? At that time, the experiments had not been done. This afternoon we have gone over a great deal of very interesting information regarding ferritin, but we are still faced with the question of whether it is actually part of the chain of cause and effect which ultimately kills an animal, or is simply a manifestation of the fact that the animal is going to die. Have you, or has anyone else, attempted to protect against shock by the administration of antiferritin serum? This would seem to me to be a most critical experiment and one which would determine whether the ferritin per se was part of the etiologic chain

Shorr I think Dr Zweifach can tell you of our few experiments in that direction, and point out what we believe are the limitations of that approach.

Zweifach A recent report by Dr Hampton (55) indicates that the administration to rats of antiferritin serum (to rat ferritin) had no protective action against the lethal outcome of the shock syndrome In our own experiments, the administration of antiferritin serum to dogs, in amounts estimated to antagonize any ferritin which might be produced or present in the circulation, gave equivocal results, chiefly because of the many untoward side effects For example, a widespread clumping or agglutination of red blood cells occurred, together with leukocytic sticking Accompanying this, there developed a considerable slowing of the circulation through the capillary bed No clear cut answer could therefore be obtained with respect to the possible protective action of antiferritin serum during the shock syndrome

Shorr: I think there are other difficulties, too, in that the antiserum, while it may be capable of temporarily clearing the blood

*For complete discussion by Dugal, see *Cold Injury* Ferrer, M I, Editor Trans Second Conf New York, Josiah Macy, Jr Foundation (To be published)

stream, cannot cope with the overwhelming ferritin content of the body We found that after early improvement, the situation deteriorates. Isn't that true?

Zweifach In experiments where the antiferritin serum was administered slowly by drip infusion, no sustained improvement in blood flow could be obtained Despite the fact that the blood appeared to be cleared of active ferritin during the infusion, VDM activity again was present in the blood stream within 15 to 20 minutes after cessation of the infusion. This may be due to the difficulty of actually clearing the tissues of active vasodepressor material by intravenous administration of antiferritin serum

Nickerson. Is there any procedure by which active immunization against ferritin could be produced?

Zweifach It is possible to immunize animals actively against heterologous ferritin, but probably not against their own ferritin I do not know whether active immunization against homologous, naturally occurring, proteins has ever been accomplished

Nickerson All you can do, then, is produce antiferritin serum, e g, by injecting rat ferritin into a rabbit, and then injecting the rabbit serum into a rat?

Cotzias Dr Zweifach, you might be able to immunize a dog against his own ferritin with some of the adjuvant techniques that Dr Freund uses

Zweifach That is a distinct possibility, but has never been tried

Burch Dr Shorr, I wonder if you would tell us how you visualize the role of ferritin in shock.

Shorr * A specific sequence of events occurs in temporal relation to the appearance of the two vasotropic factors which act in opposite directions on the terminal vascular bed The effects of VEM are to assist in the vascular compensation to reduction in blood volume, the effects of VDM are the contrary The temporal relationship between the appearance of the vasoexcitor, VEM, and compensatory mechanisms, the association of VEM with recoverability from shock by transfusion, plus the deleterious effects of exclusion of the kidney on the shock syndrome, lead us to infer that VEM may constitute an important contribution to the compensatory reaction This does not exclude other factors from operating, as undoubtedly they do

The time of appearance of the hepatic vasodepressor, its persistence in the circulation in regular association with decompensa-

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*This and other work of Doctor Shorr and his associates, reported at this Conference, has been carried out with the aid of grants from the Josiah Macy, Jr Foundation, the National Institutes of Health, U S Public Health Service, Eli Lilly and Company, and the Postley Hypertension Fund

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have shown that Noble-Collip drum resistant rats exhibit metabolic changes to a much lesser degree than do nonresistant rats Their results included data on the rise in blood amino acid nitrogen From these experiments, however, it is not possible to determine whether the conditioning is on a circulatory or on a metabolic level Thus, there is no way of knowing whether the metabolic changes did not occur because no significant tissue hypoxia resulted from the injury, or whether the tissues responded differently and were resistant to hypoxia In Dr. Shorr's experiments, apparently the liver was hypoxic and yet maintained its ability to inactivate VDM If the same is true of the liver's ability to metabolize amino acids, etc, then conceivably one could attach the same significance to these changes as to VDM, but with the important qualification that none of the metabolites under question has been shown to exert an influence on circulation, as does VDM On the other hand, if it is shown that when the conditioned rat's liver is hypoxic it still inactivates VDM but does not metabolize amino acids normally, allowing amino nitrogen accumulation in the blood, then the two phenomena have very different meaning At the moment, we do not have the data to resolve this problem

In general, with regard to the significance of the many metabolic changes which have been described as occurring during shock, one is forced to the conclusion that they are all secondary to the effects of tissue hypoxia, and there is no conclusive evidence that any one change, or group of changes, is in itself critical in determining whether irreversible circulatory failure eventuates Furthermore, while there is evidence that different organs and tissues become subjected to hypoxia at different rates and degrees, and have different sensitivities to hypoxia during shock, there is no unequivocal evidence to single out metabolic failure of any one organ as responsible for the final circulatory decompensation To be sure, somewhat more striking evidences of alterations in liver metabolism have been described, but one may question whether this is not more a reflection of the multiplicity of hepatic metabolic functions which are modifiable by hypoxia, than an indication that the liver is more seriously embarrassed than other organs by peripheral circulatory failure At the present stage of our knowledge, I think we can only say that a variety of metabolic changes eventuate as a result of the hypoxia of circulatory failure, and that in general these reflect a shift to an anaerobic type of metabolism, with consequent decrease in efficiency in terms of energy yield This latter fact in itself might be considered as the most important consequence of the initial hypoxia, and if uncorrected might be expected to lead to increasing deterioration of many essential systems and functions which are dependent on a continuing energy supply At what point these summated changes and energy deficits become critical remains to be established To me, the most important problem now from the metabolic standpoint is to determine the mechanisms involved in the phenomenon of conditioning If, as suggested by Dr Shorr, certain organs or tissues are still subjected to hypoxia after injury in the conditioned animal and yet the animal survives, it becomes most important to determine in what way the organs and tissues of the conditioned animal have modified their response to hypoxia By this approach, it

tory peripheral vascular phenomena, the profound augmentation of the VDM inactivation mechanism in animals who are protected from shock by training, the fact that Dibenzyline-treated animals who are protected from shock avoid the early appearance of VDM and the deterioration of the liver inactivating mechanism – all these persuade us that the decompensatory phase with eventual irreversibility is dependent in a very considerable measure upon what happens to the ferritin mechanisms. This, too, does not exclude the participation of other deleterious factors which may be necessary to provide for the full development of the phenomenon of irreversible shock However, it has been our experience that unless this picture of peripheral vascular decompensation has unfolded, with ferritinemia and deterioration of the ferritin inactivation system, we have not encountered irreversible shock Therefore, we regard it as an inevitable concomitant of the series of vascular events, which culminate in circulatory collapse

We recognize that many gaps exist for example, evidence of the kind that insulin provides in its relationship to carbohydrate metabolism We regard the actions of these materials as manifesting themselves, either beneficially or deleteriously, in situations which put the circulation in a precarious condition, but under ordinary conditions either of these materials may exist in the blood stream in excess and the organism will retain its ability to maintain the circulation through the involvement of all of the other adaptive mechanisms that contribute to circulatory homeostasis

Stead. Dr Engel pointed out in his discussion this morning that certain things nearly always occur with shock in his experience with rats, the plasma amino acids always rise I should like him to outline for us the differences between the appearance of the chemical changes that we think are nonspecific in regard to shock, but which always appear, and the appearance of VDM I want to know if just one system, such as the rise in amino acids, was charted across the board with the rise in VDM, where the differences would be

EDITOR'S NOTE Dr Engel added the following to the remarks he made at the Conference

> The comparison of the changes in plasma amino nitrogen levels during shock with Dr Shorr's data on VDM, is not easy to make because certain key data which Dr Shorr has about VDM are not yet available to us, with regard to other metabolic alterations during shock It is true that, by and large, a substantial plasma amino nitrogen increase occurs and persists in irreversible shock, but does not take place, or is only mild and transient, when there is either spontaneous recovery or a successful response to therapy McShan, *et al* (23),

Remington. A dog that will wag its tail, eat avidly, and play with me, is not a vegetable.

Moe: I am not so sure that tail wagging requires a very high level of cortical activity, but I could be wrong.

Fremont-Smith: The question here is twofold: (*a*) What happens to the cerebral circulation, if the head is low, would a blood pressure of 30 mm Hg be enough? (*b*) Would the survival of the animals be worth-while since their brains have deteriorated. Of course, it can be said that the beginning of any kind of therapeutic effort is not worth-while unless it provides for a guaranteed recovery, On the other hand even if these dogs were vegetables, it might still be worth-while to keep on in the hope that they could be devegetated.

Moe. You are making an extreme interpretation of my question.

Fremont-Smith: I knew you didn't mean it that way.

Moe: I was thinking that if I myself were subjected to a prolonged hypotension which destroyed my power of cerebration, I would prefer not to be alive

Fremont-Smith: I would feel the same way.

Remington: I had a long list of comments that I had wanted to make, but with the press of time I shall speak on only two points. First, may I repeat from last year that the floor under the blood pressure level seems to have been lowered in the Dibenamine-treated animal Ordinarily, when the diastolic pressure falls lower than 20 mm Hg in an animal, the manometer's calibration must be suspected With bled, Dibenaminized dogs I have perfectly adequate records of pressure values of, say, 30/8 mm Hg. I have seen dogs with cardiac indexes below that which would be lethal in an untreated dog, with mean arterial pressures of 30 mm Hg, wake from anesthesia. With pressures still below 50 mm. Hg, such an animal could walk The next day those dogs appeared to be fully recovered, and, in my terminology, they are not vegetables.

My second point is by way of a precautionary note We spent about a year trying to do direct measures of visceral flow. The results were never published, except in abstract form (56), for we were quite unsatisfied with the methodology Nevertheless, the findings were consistent, and perhaps meaningful. In the dying, control dog, and in the Dibenaminized dog having the same pressure level but not dying, the oxygen content of hepatic venous blood was of the order of 1 to 2 volumes per cent At an earlier stage in the bleeding, the oxygen content was either the same for the two animals, or tended to be higher in the untreated animal.

should become possible to differentiate metabolic alterations which are significant for survival from those which are trivial, as well as to learn something about the fundamental mechanisms of adaptation to injury

Shorr. There is one thing I wanted to add, and that is that we believe this work has some relevance to human shock I think I am permitted to quote some studies we did on seven bloods from Korea. These were frozen as soon as possible after being drawn, and shipped by plane They reached us in a frozen state, and were coded so that we did not know whether they came from normal or shock patients We assayed them and found three bloods to be free of either VEM or VDM. These proved to be from soldiers who had hemorrhaged and recovered We found four bloods to have high concentrations of VDM. Of these four patients, three died, and one survived The one that survived, the false positive, was a soldier who had received a large dose of morphine about a half hour, or an hour, before drawing the blood, a circumstance that is prone to develop vasodepressor activity Thus six out of seven predictions were correct It would seem that the same mechanisms are operating in human shock as in animal shock, and that the same predictability exists, at least to judge from this small series.

FURTHER OBSERVATIONS ON THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN SHOCK

Moe: I have always supposed that the compensatory mechanisms at the disposal of the cardiovascular system, had as their purpose the maintenance of a level of arterial pressure which would guarantee an adequate perfusion of the brain and the heart, and it has seemed to me that probably the brain is relatively intolerant to prolonged periods of hypotension If this be true, I wonder whether, in the light of these experiences with Dibenamine, it is really an advantage to survive a period of hypotension In other words, what happens to the cerebral cortex in the animals that do survive? Do they have the same cortical power, let's say, as a normal animal? Granted they are still living, but your criterion of life is the maintenance of blood pressure 24 hours later, and the persistence of respiration, and so on Are these animals much better than vegetables? Has a maze test, for example, ever been run on rats before and after a period of prolonged hypotension?

Shorr. They behave like normal rats and dogs

Moe. Yes, I dare say they do

Remington They are not vegetables, very definitely not!

Moe What is your criterion?

Burton. This would agree, wouldn't it, with our knowledge that epinephrine increases the flow in the liver? (61,62) On general pharmacologic theory, a sympathicolytic agent would, if anything, decrease the flow then, wouldn't it?

Nickerson. I would arrive at a somewhat different conclusion. The adrenergic blocking agents with which we are acquainted block only the excitatory, constrictor effects. They do not alter the inhibitory response. If the change in liver circulation in response to epinephrine is a composite, as is the case in most vascular beds, with some constriction and more dilation, and the constrictor component is blocked, the dilation would appear to be even more pronounced.

Fremont-Smith: Is there any information about the influence of these drugs on cerebral blood flow? It would seem to me that if there were any possibility of a cerebral vascular dilation of arterioles, then there would be a basis for survival at a level other than the vegetable at a considerably lower blood pressure, because the rate of delivery of oxygenated blood to cerebral capillaries, which is the key point, would be kept up.

Nickerson: I do not know of any studies with pressures as low as 30 mm. Hg, but at higher pressures cerebral blood flow stays relatively constant even though the arterial pressure is lowered by drugs (63). The same thing occurs when the blood pressure is reduced by spinal anesthesia not high enough to denervate the cerebral bed (64). It appears that the cerebral circulation, and to a large extent the renal circulation, takes care of itself, that is, internal compensation allows the cerebral vessels to dilate as the blood pressure goes down. There are undoubtedly limits to this compensation, but they have been inadequately studied in patients with a normal circulation.

Fine. We* have made studies on the sagittal sinus during arterial pressures as low as 30 mm. Hg.

Knisely. There is a point here of straight physics. I can't tell you quickly the Poiseuille equation (65), but, if I remember rightly, the volume flow through a small tube is proportional to the total pressure drop, and proportional to the fourth power of the radius. Thus, you could have a large drop in pressure compensated for by a very small increase in radius.

Nickerson. There is, of course, considerable argument as to just what role the sympathetics play in the control of the cerebral circulation. Forbes (66) and others, years ago demonstrated that

*Fine, J., Frank, H., and Frank, E. Unpublished data.

The results from the flow determinations indicated that, if there was any real difference, the liver flow was lower in the Dibenamine-treated dog. Of various regional flows measured, the only clear difference between the two animals was with the hind leg flow, where the flow was not curtailed to nearly the same extent in the animal given Dibenamine Presumably, this would apply for other muscle beds

Burton Did I understand you to say it is reduced through the liver?

Remington It is seemingly reduced through the liver

Burton. A lot of the previous discussion implies an increase in the flow

Remington No, it is reduced

Selkurt What was your method for measuring this?

Remington We tried various procedures and were dissatisfied with them all First, we tried the Blalock technique of passing a brass pipe, bearing a balloon, down the jugular vein into the inferior cava Inflation of the balloon prevented venous return from the abdominal cava and allowed collection of hepatic venous blood However, any pipe we could get into the jugular vein could not carry enough flow to support the animal The same was true of a larger pipe introduced into the inferior cava through a slit in the auricular appendage We then turned to a rotameter in the inferior cava, above the hepatic veins, or one in the portal vein In all cases, the course of circulatory events after the rotameter was inserted was quite different from that seen in an intact dog subjected only to hemorrhage What we were actually studying was a mixture of hemorrhage and "rotameter," or venous resistance, shock (57,58) On the other hand, the directional pattern of flow change was consistent, and there was no great change in resistance of the liver circuit as the animal failed

Selkurt: This must be in the Dibenaminized animal

Remington. In the control, too

Shorr Isn't that inconsistent with Wigger's findings?

Selkurt It is inconsistent with our findings (59) and also Wigger's (60)

EDITOR'S NOTE Dr Remington wished to add the following "afterthought" at this point

> It should be pointed out that the visceral flows we measured were in dogs bled stepwise until death, and that no transfusions were given In general, the high hepatic resistance reported from Cleveland applies to the post-transfusion picture rather than to the pretransfusion hypotensive phase

Stead I am just pointing out that these people think well. I am giving a compliment to the group

RELATION OF BACTERIAL ACTION TO DEVELOPMENT OF STATE OF IRREVERSIBILITY IN SHOCK

Shorr. A year ago Dr Fine told us about work going on in his laboratory which made us realize that we were going to have to recognize the participation of new factors in the shock syndrome I am sure that he must have a lot more to tell us now

Fine For the benefit of those who were not here last year, I shall repeat some details concerning the technique we use for control data, upon the basis of which we make observations on the effect of the bacterial factor in hemorrhagic shock

The dog is healthy as far as we can judge: free of fever and of any obvious malnutrition It is well fed for at least a week before being subjected to blood loss Bleeding is started an hour or so after it has been given a small dose of morphine to reduce apprehension Sometimes the morphine is omitted Novocain is put into the groin, the vessels are cannulated, and the artery is connected to a reservoir containing heparin, elevated so that bleeding will stop when the blood pressure falls to 30 mm. Hg systolic, where it remains without any fluctuation for some hours That level was selected because we had found by experience that a level below 30 would produce untoward cerebral responses, changes in respiratory rate, convulsive seizures, and so on But at 30 the animal is responsive indefinitely, at least until the time when irreversibility sets in We selected this level deliberately in order to produce the most drastic form of hypotension consistent with recovery when transfused

The animal is allowed to take back the blood from the reservoir as it requires it Observation demonstrates that during the first 45 minutes or so the dog bleeds into the bottle, so that the volume in the bottle increases and eventually reaches a maximum of about 53 ml per Kg body weight There is very little variation in maximum bleeding volume from dog to dog, not more than a few per cent in the entire series About an hour after the bleeding has been started, the dog begins to take back some of the blood If the dog is transfused with what is left in the bottle before it has spontaneously taken back two-fifths of it, it will recover in most instances By recovery, I mean that the next day, the following day, and thereafter, one can't tell such an animal from one that has not been submitted to the experiment In behavior, it is normal It eats, is

it is possible to obtain cerebral vasoconstriction by stimulating the cervical sympathetic nerves However, it is very much less than the constriction obtained in other areas of the body. Consequently, blocking these sympathetic nerves might not have a major effect.

Stead: Actually, the maintenance of blood pressure, even in man, doesn't seem to be terribly important at certain times. It is rare to see any symptoms of cerebral dysfunction develop in a person with neurogenic postural hypotension as long as the systolic pressure remains over a level of 50 mm Hg This is remarkable when you consider that blood must be raised against gravity from the heart level to the head. Of course, this differs from the person without sympathetic paralysis, in whom the pressure is maintained for a long time during motionless standing but finally drops precipitously

Burton. On this question we need to distinguish carefully between reversible and irreversible damage to the brain. We know that if the oxygen supply to the brain is completely cut off, even for as short a time as 30 seconds, it may result in damage to the cells which a pathologist can find On the other hand, reduced blood flow may lead to unconsciousness but may not produce any irreversible damage which will give cerebral symptoms later

I have always been bothered about this question of sympathectomy giving some protection in shock to the animals in our laboratories It seems in such violent conflict with Cannon's idea of fight and flight, and the role of the sympathetic nervous system which is to preserve the animal in fight However, there isn't any real conflict, because, I suppose, in nature a small drop in the blood pressure will make the animal unable to win the fight. Conditions in the laboratory and in the hospital are utterly different The blood pressure, then, I think, becomes of very little importance to the brain as long as there is enough flow to prevent irreversible damage It helps me to resolve that theoretical biological difficulty in that way

Stead There is certainly no question but that these people don't fight well and if put to chopping wood, they faint away, but the fact remains that they can supply the brain and maintain a normal metabolism at an extraordinarily low blood pressure

Burton What would happen if they were put in a conference such as this?

Stead: They would be, as far as we can tell, just as good as any of the gentlemen around the table

Fremont-Smith· On whom are you casting aspersions?

Stead I am just pointing out that these people think well. I am giving a compliment to the group

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frisky, and can later be used for other experiments. However, if the dog is transfused after it has taken back more than 40 per cent of the maximum loss, the recovery rate is reduced to 1 in 5 or less. There is a pressor response to the transfusion, but it does not last very long. After the pressure falls to 60 systolic, it goes down precipitously and the dog is dead in an hour or so.

This is the standard experiment It shows that a dog can tolerate a blood pressure of 30 mm. Hg for an average of 4 8 hours without noticeable permanent damage, if its blood loss is fully restored before the end of that period of time The animal has had no drugs of any kind except one dose of morphine, which does not influence the result one way or the other In other words, we are able to produce a state of irreversibility to transfusion in the unanesthetized dog provided we subject it to this degree of hypotension for longer than an average of 4 8 hours before fully replacing its blood loss

After many years of studying this problem, we came to the conclusion, as Dr Engel also believes, that one cannot attribute the development of irreversibility directly to functional failure of any vital organ We worried about such things as electrolyte imbalance, and we satisfied ourselves that maintenance of electrolyte balance, by use of the artificial kidney, did not prevent the development of irreversibility So we began thinking all over again about the possibility of irreversibility being related to bacterial action

Shorr Wasn't there one organ that you did implicate through your viviperfusion experiments?

Fine Yes, but we were stumped While we thought we were doing something that indicated that the liver was rather important, we couldn't interpret its meaning

Shorr Would you describe those experiments for us?

Fine The liver was subjected to cross circulation with a donor animal, so that the liver of the recipient, whose systemic arterial pressure was kept uniformly at a level of 30 mm Hg as described, received from the donor animal some 400 ml. of arterial blood per minute, an equal amount being taken from a vein and returned to the donor animal If perfusion was started at the beginning of the hypotensive period, the animal was responsive to the return of blood many hours afterward, much longer than the control dog. This protective effect was not obtained when we perfused the recipient via a systemic vein The liver, therefore, was certainly involved in a very important way, but we do not know how. Dr.

Shorr also feels that the liver was important. So do Dr Engel and Dr Long But we cannot nail down the factor of importance

All of us are familiar with the fact that if the liver is taken out entirely, rapid disintegration ensues, but it is not nearly so rapid as it is in the case of a deeply shocked animal We eventually came to the conclusion that something was developing in the unprotected liver that was deleterious It might be VDM or it might be something else Hence, we decided to study the bacterial factor, because if results varied in different species, it might be due to differences in the bacterial content of the liver and other tissues

We had long before this tried antibiotics by putting penicillin into the reservoir, directly into the circulation, and injecting it intramuscularly during the shock period. But there was no benefit of any consequence Then we thought that since penicillin has a limited range, we should try Aureomycin With Aureomycin given intravenously, we didn't get much farther Instead of the average recovery rate of 5 or 20 per cent in the control series, we got a 40 per cent recovery rate, but this was not definitive enough to assess the significance of the bacterial factor

In the meantime, we had been doing work on the bacterial factor in such disorders as pancreatitis and in peritonitis developing in patients whose peritoneal cavity was being irrigated for the treatment of uremia In this work, evidence was obtained showing invasion of the peritoneal cavity by intestinal bacteria It seemed to us that the same might occur during shock as a result of anoxia in the gut Because we were able, in some of our experiments on pancreatitis and peritonitis, to prevent death if an antibiotic was given orally but not parenterally, we repeated the shock experiments with the antibiotic administered orally We began with Aureomycin, and for the first time, there was a really substantial difference in the mortality rate In the first 25 dogs to which were given Aureomycin, there was an 88 per cent recovery rate Moreover, these animals could tolerate the hypotensive periods for much longer periods of time Instead of an average period of 4 8 hours of tolerance for the 30 mm level of pressure before they became decompensated, we found that the dogs fed Aureomycin could go eight hours and still respond to transfusion They sometimes drank water within an hour after they got the transfusion, and the next day they were frisky, and seemed none the worse for the experience

I should like to emphasize the fact that every time an experiment with an antibiotic was done, we always ran a concurrent control

animal without the antibiotic. Other antibiotics were tried in order to discover what organisms were involved. Before exploiting the other antibiotics, we investigated Clostridia, which are the predominant bacteria in the liver of the dog. If the Clostridia in the dog's tissues have any bearing on the phenomenon of irreversibility, why should Aureomycin, which suppresses the Clostridia in the gut, not do so when given intravenously? We tried clostridial antitoxin, in huge amounts for days. We gave it before we induced shock, and during shock, but we made no significant impression on the survival rate. It was not more than 30 per cent. Then we used the toxoid, which Dr. Logan and Dr. Altemeier of Cincinnati developed, and which they consider able to produce immunity to the Clostridia. It did not make any impression either. I shall leave the question of the Clostridia for further reference, after I describe the series of experiments which were done with other antibiotics, to see which other intestinal flora, if not the Clostridia, might be responsible.

Neomycin was then tried orally, because this drug does not reduce the Clostridia in the gut, and, moreover, only three per cent of it is absorbed. (At the time we thought none of it was absorbed.) It was as effective as Aureomycin. We then tested Terramycin, and got about an 80 per cent recovery rate. It was surprising that there was almost no therapeutic benefit from chloramphenicol, which is an absorbable drug with a considerable antibacterial range. Bacitracin, which is nonabsorbable, was tried next. In spite of the fact that this drug has a remarkably rapid effect on the intestinal flora, there was no substantial improvement with it either. Streptomycin produced a better recovery rate, about 50 per cent, but this was not nearly as striking a result as with the broader-range antibiotics. From Sulfathalidine there was no benefit.

In summary, we received no clue whatsoever from these results as to which bacteria were involved, and we wondered whether we were getting an effect which had anything to do with antibiotic action. From our first results with the oral route, we assumed that intestinal bacteria were invading during the shock process. The result was not good by the oral route when we gave the drug a few days in advance, unless we added a large priming dose on the morning of the experiment. It was apparently necessary that the dog's intestine be thoroughly saturated with antibiotic when the experiment began. Priming alone, while not nearly as effective, was still very substantial in its effect. We then found that while the antibiotic, when administered intravenously after the dog was in

shock, was not very effective, a good result was obtained with neomycin and Aureomycin when given via the portal vein for several hours before bleeding was started We concluded that while giving the drug orally, especially during the few hours preceding the precipitation of shock, probably reduced the degree of bacterial invasion during the shock phase, the oral route was not essential The essential thing was the deliverance of an effective antibiotic to the site of greatest bacterial action, namely, the liver, under conditions when it could get there. Apparently, the drug could not get to the site of action as well if the antibiotic was given only after shock had been precipitated, or when given via a systemic vein rather than by mouth or via the portal vein.

I should like to show you now the actual data

In Table II the top figure in the righthand column, 21 per cent, is the average survival rate in 201 control animals If a definitive effect of an antibiotic is arbitrarily limited to 65 per cent or better, there is not much doubt that with certain antibiotics a positive effect is obtained upon irreversibility to transfusion Bacterial species, sensitivity, or absorbency of the drug cannot be correlated with the effect of the antibiotic But we can say, in general, that the only antibiotics which prevent irreversibility are among those which have a broad range and which are absorbable (excepting neomycin, which is poorly absorbed)

This being so, whether the dose used was in fact an antibacterial dose or a pharmacologic dose, will have to be considered With penicillin, there was a 65 per cent recovery rate when given through a duodenal catheter prior to inducing the shock, and continued parenterally during the shock period (Table II, 5 a) Penicillin has no pharmacologic action As for Aureomycin, we used 5 gm as a priming dose (1A a.) That is a huge dose for a dog I do not know whether this dose signifies a phamacologic action Since we got an equally good result with neomycin in the same dose (3 a and b), and only three per cent is absorbed, I would say no Moreover, the dose of both drugs via the portal vein was only 500 milligrams

Shorr What do you mean by "a pharmacologic action?"

Fine I would say a nonantibacterial action

Fremont-Smith Other than an antibacterial action?

Fine Yes

Shorr Would you describe the experiment using inactivated Aureomycin?

Fine That is the next point I should like to mention We thought inactivation of the Aureomycin should be tried. We did this by dissolving it in normal saline solution, exposing it to a temperature of 37° C for four days, and then evaporating to dryness We assayed the dried material and found that its antibacterial action was reduced to between 0 0001 per cent and 0 001 per cent of that of the active drug With this material, the result was not the same as that obtained with the active drug (compare 1A b. with 1B.j and 1A h with 1B k, Table II)

Shorr. Wasn't the survival 61 per cent?

Fine It was 61 per cent at 36 hours, but only 38 per cent permanent survival The figures I am talking about, and which I consider valid, are those for permanent survival without any damage that can be identified from observation of the behavior of the animal

We wanted to try other inactivated antibiotics, but did not know how to inactivate them Inactivated penicillin has not yet been tried but we have been told that penicillin inactivated by penicillinase is no longer penicillin We do not yet know how to test the matter further The problem, then, as to whether this is a pharmacologic or an antibiotic action, has not been definitely settled, but I think the evidence is rather in favor of an antibiotic action

Going further into the mechanism of action of the antibiotics. since they do not have to be given so as to act upon the bacteria within the gut, helpful as that seems to be, we wondered what their effect was upon the bacteria in the tissues Accordingly, routine blood cultures were done throughout the shock period In Table III, data on cultures of blood taken from the systemic veins, the portal vein, and the lymph of the thoracic duct are given The results are almost uniformly negative in both treated and untreated dogs The blood for these cultures was taken in various ways a drop at two minute intervals, or by continuous flow with replacement by donor blood In several instances, we exsanguinated the animal and cultured an aliquot

Shorr These are your control animals without antibiotics?

Fine Both types, treated and untreated The results, for all practical purposes, are uniformly negative

Table IV shows data on cultures from animals that died, from those surviving antibiotic therapy, and from normal dogs The cultures were made immediately after death or sacrifice The portal blood shows bacteria, although in smaller incidence The peritoneum also has some, but the liver yields positive cultures in

TABLE III[1]

Blood and Lymph Cultures During Hemorrhagic Shock

Group	No of Dogs in Group	Source of Specimens from Each Dog	INCIDENCE OF POSITIVE BLOOD AND LYMPH CULTURES							
			Control Before Bleeding	Hypotension Before Transfusion (hours)				Post-Transfusion Period (hours)		
				1-2	2-4	4-6	6-8	¼	1-2	2-4
1	10	Portal vein	0	0	1 (Cl)	0	0	1 (Cl)	0	0
		Vena cava	0	0	0	0	0	0	0	0
		Aorta	0	0	0	0	0	1 (Ps)	0	0
2	3	Portal vein	0	(------	-----0-----	------)	—	(------	-----0-----	------)
		Vena cava	0	0	0	0	—	0	0	0
		Aorta	0	0	0	0	—	0	0	0
3	2	Portal vein	0	—	—	2 (Cl)				
		Aorta	0	—	—	0				
	3	Portal vein	0	—	—	—	—	—	—	0
		Aorta	0	—	—	—	—	—	—	0
4	3	Thoracic duct	0	(------	-----0-----	------)	—	(------	-----0-----	------)

(Cl = Clostridia)
(Ps = Pseudomonas)
(Diphtheroids, subtilis, sarcinae or non-hemolytic staph albus were found in 12 cultures)

[1]This Table appeared in a progress report submitted to the Subcommittee on Shock of the National Research Council under a contract with the Office of Defense and is reproduced here by courtesy of the Office of the Army Surgeon General

TABLE IV[1]

Cultures of Blood, Peritoneum and Liver Taken Immediately after Death

GROUP		No of Dogs in Group	POSITIVE CULTURES (Percent of Group)							
			Portal Blood		Caval Blood		Peritoneum		Liver	
			aerob*	an-aerob	aerob*	an-aerob	aerob*	an-aerob	aerob*	an-aerob
NO SHOCK	Sacrificed	20	10	25	10	0	0	16	5	100
SHOCK (NO ANTIBIOTIC THERAPY)	Survivors†	18	11	22	0	17	22	17	39	89
	Non-survivors	43	23	30	14	33	14	37	60	95
SHOCK (ANTIBIOTIC THERAPY)	Survivors†	123	7	24	4	16	11	35	22	94
	Non-survivors	111	24	41	15	35	21	39	37	78

*In 353 positive cultures (200 single, 153 mixed), aerobic organisms were found in the following proportions

Pseudomonas	41%
B Proteus	22
E coli	19
Enterococci	18

†Sacrificed in good health 48-72 hours after shock experiment
75 contaminants (diphtheroids, subtilis, non-hem staph albus) were found in 1100 cultures

[1]This Table appeared in a progress report submitted to the Subcommittee on Shock of the National Research Council under a contract with the Office of Defense and is reproduced here by courtesy of the Office of the Army Surgeon General

practically every case There seems to be a higher incidence of positive cultures in the nonsurvivors, but the differences are not significant In other words, although we have apparently succeeded in curing the animal of shock, we have not succeeded in sterilizing its tissues

Shorr Could you tell us about the number of bacteria per milliliter?

Fine. No We have no quantitative data These are biopsy and fluid specimens cultured on blood agar plates, in thioglycollate and in thioglycollate broth, and both anaerobically and aerobically In addition to Clostridia, these animals show, in much lower incidence, other types of organisms, but they are intestinal flora in every case There is about a 20 per cent incidence of non-clostridial bacteria, such as Proteus, Pseudomonas, E coli, etc It is remarkable that immediately after death one gets positive blood cultures. The blood of these same animals sampled very shortly before death is sterile

Knisely The speed with which bacteria can be removed from the blood is fantastic The rate of multiplication of the organisms may be very fast Since they are taken out rapidly during life, very few will be found at any one time until after death, and because they continue multiplying during and after death a great many will be found *after* death

Fine. Yes, the conclusion from these data is that something happens to the antibacterial properties of the plasma at the moment of death which allows one to grow out bacteria that are presumably present in life but are not capable of growing out in culture so long as they are taken from blood which is still circulating This suggests that, at least in the dog, there is a continuous invasion of the blood stream

Whether the bacteria in tissues of the dog in shock are more pathogenic than those of the normal dog is perhaps answered in the data of Table V Homogenates of liver from the animal in shock were implanted in the peritoneal cavity of guinea pigs, which are ordinarily free of Clostridia Similar experiments were done with liver from healthy dogs Notice that the number of guinea pigs, which died when the amount given was 3 to 5 ml, is far greater in the case of the liver in shock than it is in the case of the normal liver This suggests activation of these bacteria in the liver of the animal in shock Data on untreated animals which died in irreversible shock are given in the Table The dead guinea pigs showed Clostridia in the liver, which is ordinarily free of them It is, there-

TABLE IV[1]

Cultures of Blood, Peritoneum and Liver Taken Immediately after Death

GROUP		No of Dogs in Group	POSITIVE CULTURES (Percent of Group)							
			Portal Blood		Caval Blood		Peritoneum		Liver	
			aerob*	an-aerob	aerob*	an-aerob	aerob*	an-aerob	aerob*	an-aerob
NO SHOCK	Sacrificed	20	10	25	10	0	0	16	5	100
SHOCK (NO ANTIBIOTIC THERAPY)	Survivors†	18	11	22	0	17	22	17	39	89
	Non-survivors	43	23	30	14	33	14	37	60	95
SHOCK (ANTIBIOTIC THERAPY)	Survivors†	123	7	24	4	16	11	35	22	94
	Non-survivors	111	24	41	15	35	21	39	37	78

*In 353 positive cultures (200 single, 153 mixed), aerobic organisms were found in the following proportions

Pseudomonas	41%
B Proteus	22
E coli	19
Enterococci	18

†Sacrificed in good health 48-72 hours after shock experiment
75 contaminants (diphtheroids, subtilis, non-hem staph albus) were found in 1100 cultures

[1]This Table appeared in a progress report submitted to the Subcommittee on Shock of the National Research Council under a contract with the Office of Defense and is reproduced here by courtesy of the Office of the Army Surgeon General.

shock state is produced, because it must be available during the stage of activation of the bacteria. Penicillin is no good for an infestation unless given while the organisms are growing We gave dogs large amounts of antibiotics after irreversibility had developed. They were not benefited in the least, presumably because a sufficient amount of toxin had already been elaborated, or because the antibiotic could not reach the site of bacterial action in sufficient concentration owing to deficient flow in the capillary circulation What this points to by way of further study is, of course, obvious

I should like to mention one more thing In the light of these newer data, we should review the data on tourniquet shock gathered during the war in order to understand why tourniquet shock in the dog is so much more irreversible to blood volume therapy than hemorrhagic shock It may that we can explain it in terms of the extensive infection in the muscles induced by the tourniquet. I think this may also explain the source of the vasodepressor substance which Dr Shorr found in the blood leaving the extremity the minute the tourniquet was removed

EDITOR'S NOTE:

> Dr Fine has reported that since the Conference, his group has found that clostridial antitoxin prevents the death of guinea pigs following implantation of liver homogenate from dogs in shock Also that preliminary observations on the rat, carried out since this Conference, show that irreversible hemorrhagic shock can also be prevented by oral antibiotics in doses within accepted therapeutic range, i e , 150 mg. per kilogram Invasion from the intestinal or respiratory tract is the obvious inference

Shorr We considered that possibility and believe that we have ruled it out We simulated the hypoxia these limbs are exposed to, by incubating muscle anaerobically *in vitro* under sterile conditions Bacterial counts were done for us by Dr Rene Dubos as a check on the technique A vasodepressor was released, as usual.

Fine: Dr Haist has some interesting and pertinent data on tourniquet shock in the rat

Nickerson Before we go on, I should like to go back for just a moment to the experiments with the inactivated Aureomycin. I was very much impressed by the degree of survival which Dr. Fine obtained The 36-hour survival increased from 24 per cent to 61 per cent, and although the over-all survival increased less, it still almost doubled. This was with a preparation which had only 0 00001 of the antibacterial action of the original Aureomycin It was given in the same dose as the active Aureomycin To me, it is amazing that the "antishock" property of the Aureomycin

TABLE V[1]

Intraperitoneal Injection of Liver Tissue Suspensions into Guinea Pigs

Test Dose Liver Homogenate (cc)	SHOCK LIVER		CONTROL LIVER	
	Number survived	Number died	Number survived	Number died
¼-1	6	0	8	0
2	8	1	8	0
3	10	2	9	1
4	6	6	11	0
5	3	5	6	2

"Shock Liver" from dogs in irreversible hemorrhagic shock
"Control Liver" from normal dogs

[1]This Table appeared in a progress report submitted to the Subcommittee on Shock of the National Research Council under a contract with the Office of Defense and is reproduced here by courtesy of the Office of the Army Surgeon General

fore, conceivable that the animal in shock is being fed a poison from his own liver, probably derived from these or other bacteria because antibiotics inhibit the lethal effect.

Shorr. How soon does death occur in the guinea pig?

Fine Within 24 or, at the most, 48 hours

Oliver. Is it a death from infection?

Fine Yes, at least some of them showed peritoneal hyperemia and Clostridia locally and in the liver We concluded that the irreversibility which developed in the shocked animal was due to bacterial action

The thing to do next is to see whether irreversibility will occur when there are no bacteria in the tissues. Data from animals free of bacteria, such as the rat, can give us information In substance, since an antibiotic in the proper dose and at the proper time prevents irreversibility, failure to recover bacteria from the blood stream is insufficient evidence to exclude bacterial activity or invasion during shock The bacteria at fault are from the intestinal tract, though not necessarily those which may be invading during the shock period Giving the drug in advance via the intestine may have a better result than giving it parenterally during shock, perhaps by cutting down invasion before, as well as during, the shock process. But the antibiotic must also be present at the time the

intrathecally, it will cause convulsions. Presumably, the action involved is not pharmacologic, but until we know more about what action we are dealing with, it is impossible to say a drug does not have it

Fine. How, then, will we explain the effect of neomycin, of which only a very small fraction is absorbed from the gut?

Nickerson. I do not know, but since we are hypothecating, it seems to me quite reasonable to suggest that neomycin may have this nonantibacterial property to a very high degree Perhaps three per cent absorption is enough

Fine I talked to Professor Florey about this in September He said that, so far as he knew, penicillin in any dose has no known action except an antibacterial action *

Shorr. How does it exert its antibacterial action?

Fine I don't know

Burch It will produce immune reactions, such as urticaria

Fine Only in a few individuals

Shorr. In the last analysis the interference by all antibiotics with the growth of organisms is an interference with some biochemical process You cannot exclude the effect of these agents, particularly in these large amounts, on tissue enzymatic processes of a character similar to those which are affected in bacteria I think the point that Dr Nickerson made is very well taken, that it might be important to inject very much larger amounts of inactivated Aureomycin

The second question I should like to raise is how do the animals that survive for 36 hours, die? Do they experience the same sequence of events that we observe in animals who become irreversible to transfusion?

Fine I do not know We did not examine them

Shorr How about their blood pressure?

Fine We did not measure it

Shorr Then you really cannot say that they are dying of shock It could be something else That is why I believe that the survivals with the inactivated Aureomycin acquire potential significance

Fine Chloramphenicol, which is supposed to be an antibiotic of considerable potency and almost as good as the other three, does not give as good a result

Nickerson Is not that in itself an argument against this protection being strictly due to an antibacterial action?

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*Personal communication

survived to the extent that it did after the treatment to which the material was subjected Some reduction in this activity as compared to the fresh Aureomycin is to be expected When you alter a molecule, you cannot necessarily expect to alter only one of its properties I should certainly like to see figures on what would happen if, say, three times the dose of the inactivated Aureomycin were given, assuming that just a quarter or a third of the antishock activity was left. It seems to me that this experiment of Dr Fine's is most important and enlightening If one can, essentially, completely inactivate the antibacterial action and still have a third or, in the 36-hour survival, two-thirds of the protective action of the active Aureomycin, that is extremely strong evidence that an action other than the antibacterial action is involved

Knisely. An impossible but serious and friendly question is there any chance that the body would reactivate the Aureomycin?

Fine I wouldn't know

Knisely Is that too foolish a question to ask?

Fine I don't know, I cannot answer it

Moe But the question here is what is the significance of the data? The survival rate of untreated controls was 21 per cent of 201 animals How many animals were treated with this inactivated Aureomycin, and how significant was the 38 per cent? Dr Fine, did you regard it as significant?

Fine There were 21 animals in group 1B j and 24 in group 1B k We decided that the only standard that we could use to determine the protective action of an antibiotic was permanent survival We pondered over the rather striking result at 36 hours, but we felt that if the animal was not permanently cured of his shock, that substance was not strong enough to inhibit permanently the action of activated bacteria If we insist on a nonantibacterial explanation, we have to explain why penicillin, which is known not to have a pharmacologic action, did as well, or decidedly better, with a permanent recovery rate of 65 per cent

Nickerson That cannot be explained on the basis of what we now know about its pharmacology

Fremont-Smith· How many animals did you have with the inactivated Aureomycin? That was the question

Fine We had a sufficient number· about 20

Nickerson One difficulty is in our interpretation of a pharmacologic action I do not agree with the implication that bacteriostasis is not a pharmacologic action Penicillin certainly does have, if I may use the term in quotes, "pharmacologic actions" Given

Nickerson: I am still quite impressed with the fact that you certainly have done something to these animals with the inactivated Aureomycin

Fine I should still not want to say it is a nonantibacterial activity, because there is some activity even though it appears to be slight

Nickerson. The fact that protection with the inactivated material is not equal to that with the untreated Aureomycin seems to me quite unimportant until it has been demonstrated that larger doses will not give equal protection It would be surprising if four days of incubation did not alter several properties of the Aureomycin

Shorr. I should like to reiterate the point that antibiotics do not act in a vacuum. They act on cellular metabolism. They will act preferentially on bacterial cells, but in the amounts that we use they can also be expected to have the same type of activity upon the metabolic processes of the tissues of the body.

Cotzias That was demonstrated by Dr. Umbreit (70) with mitochondria, using a number of systems that I cannot recall If the mitochondria were intact, there was no inhibition or interference on addition of streptomycin. If the mitochondria were injured, there was some interference. The evidence was quite striking It was apparently a phenomenon of lack of diffusion of the agent into the enzyme systems No difference between the mitochondria and bacteria was shown, other than in permeability

Fine There is the question of what is an effective drug. Chloramphenicol produces a survival rate of 42 per cent at 36 hours Is that a positive result? I would prefer to say that it is at least doubtful.

Shorr I suspect that your 36-hour survival period relates directly to the shock episode and that you may be losing a valuable clue if you do not take that as indicative of a positive beneficial effect from the inactivated antibiotic.

Fine Well, I don't deny that it may be a positive beneficial effect As I said before, inactivated Aureomycin is not totally inactive It is as inactive an we can make it, and I do not know what the minimal effective dose of the active drug is Clinically, we give a patient one gram of Aureomycin a day to prevent or to cure an ordinary infection I heard the other day from Schwachman that a long term therapeutic effect in children with chronic infection can be obtained with much smaller doses Half a gram is as effective as two grams, so far as he can tell If so, perhaps a tenth of a gram would be as beneficial as two grams.

Fine: I do not know I am not willing to say that it is. It may be a question of the sensitivity of a bacterial flora that we cannot identify In our experience, chloramphenicol is not as effective as Aureomycin in surgical infections It depresses the bone marrow. I do not know what that has to do with it, either. I prefer to let the interpretation come from further studies with more antibiotics or let it stand until somebody can demonstrate that these drugs have some effect other than an antibacterial action.

Fremont-Smith. It seems to me perfectly evident that you cannot decide by argument whether or not this is an antibacterial action There is an action, there is no question about that. The survival rates are dramatic, and whether you interpret it one way or the other seems to me to be largely a matter of personal inclination, it cannot be determined in any way by the evidence at hand However, it might be quite worth-while to spend some time deciding what would be a critical experiment to do at this point or what series of experiments might bring a critical understanding.

Fine. It is surprising that more work has not been done on the action of these compounds apart from their antibacterial action All we have is the observation on the growth factor in the mash fed to pigs But that is a long-term effect.

Nelson· It is well-known now that Aureomycin has a direct plague antitoxin activity (67).

Nickerson: We really do not have to go much further than Dr Fine's experiments I think he has demonstrated a rather clearcut pharmacologic action of Aureomycin after its antibacterial activity has been destroyed (68,69) *

Fine It is almost inactivated, but not entirely We do not really know what the minimum therapeutic dose of an antibiotic is.

Nickerson. If this material is 0 01 per cent active, the amount of active Aureomycin given would be 50 micrograms. This could hardly protect the animals

Fine. Remember that this inactivation is measured in terms of an *in vitro* bioassay, which is a pretty crude method I should just like to repeat that for us the test of survival was permanent survival, and in this respect if inactivated Aureomycin is compared with activated Aureomycin, there is a tremendous difference. That has to be borne in mind

* Aureomycin is known to alter oxidative phosphorylation markedly in both liver and kidney tissue It is quite possible that the antishock properties of this antibiotic are due to this or some similar metabolic action completely unrelated to bacteriostasis

Fine I am asking too much, as a matter of fact What I am chiefly concerned with is the general question of whether the action of the antibiotic is related to the development of irreversibility While I am lost in trying to answer the question of how the antibiotics act, the data as a whole nevertheless demonstrate that antibiotics do have some relation to irreversibility That is the main point, and, I think, the most important point, to get out of these data

von Euler I should like to ask whether there is any explanation of the fact that the treatment with Aureomycin in the portal vein concurrent therapy, gives a permanent survival of 65 per cent, and that with neomycin in the portal vein concurrent therapy only 16 per cent (Table II, 1A i and 3 g) Is that due to any different action in principle, or could it have anything to do with some different action in Aureomycin?

Fine "Concurrent" means beginning the delivery of the antibiotic after shock has already been induced This usually does not provide a sufficient concentration at the site of action because capillary circulation is deficient The result with Aureomycin is certainly better This is the only instance of a good result with concurrent therapy This fact is of importance in deciding the question of antibacterial versus nonantibacterial action of the antibiotic If a nonbacterial action is the explanation, the results should be at least as good, when the drug is given by a systemic vein, as it is by the portal vein or the gut Indeed, one might expect a better result, since the liver usually inactivates drugs

von Euler That is why I just wondered whether it had anything to do with some unspecific, or, as Dr Nickerson said, pharmacologic action

Burton Dr Fine, I think last year you told us about an experiment in which you excluded the intestines from the circulation I was very much struck by that Has that been confirmed by further experiments?

Fine I have something further to say about that We took out the intestines in various ways, and during the early experiments, observed that the animal could tolerate the hypotensive period for a longer period of time As we kept on repeating the experiment, trying to get a good preparation, we found that we could not avoid the development of peritonitis, and so we gave up the idea of getting a preparation of an animal without the gut Thinking that we would try to get rid of the bacteria in the intestine in another way, we exteriorized the esophagus in the neck, divided it, and fed

I think perhaps the thing to do is to try to uncover some kind of pharmacologic action of these drugs. That is not my field. I should like to see somebody else do it

Nickerson: These experiments should provide an answer One would not necessarily expect to find, say, a sympathetic blocking action or a cardiac action The pharmacologic action which is indicated is protection against shock

Fine. How do we first get rid of the antibacterial action completely? I do not know how to do that

Nickerson That may be an extremely difficult thing to do if you are down to 0.01 per cent.

Fine The reduced activity was from 0.01 to 0.0001 per cent of the active material by bioassay *in vitro* This is very rough.

Nickerson. About the only way to get much lower than 0.01 per cent is to ash the material, which destroys everything

Fine That doesn't do anything

Nickerson: Perhaps the way to get around this problem is to compare the action of a gram of Aureomycin that has been inactivated with the equivalent antibacterial activity of active Aureomycin That is, compare one gram of inactivated Aureomycin with 100 micrograms of the active material and see if they are comparable

Fine. They are not One hundred micrograms did not seem to be good enough

Nickerson That would be very much more antibacterial activity than you would have in even your most poorly inactivated preparation

Stead. By "not good enough," do you mean it had no effect at all?

Fine No, I do not mean that We did not get what we could call a distinctly beneficial action

Stead. If the survival rate in the treated animals was significantly greater than in the controls, one cannot say that the behavior of the controls and the treated animals was the same

Fine. Yes, it is true that the figures in the control series were very consistent

Stead Did you have any survivals above 30 per cent in any control group of 24 animals?

Fine: Controls, no

Shorr. You feel that with so little variation in the control series, Dr Fine may be disregarding clues by asking too much from his therapeutic series?

Stead: That is standard deviation.

Fine. I am asking too much, as a matter of fact. What I am chiefly concerned with is the general question of whether the action of the antibiotic is related to the development of irreversibility. While I am lost in trying to answer the question of how the antibiotics act, the data as a whole nevertheless demonstrate that antibiotics do have some relation to irreversibility. That is the main point, and, I think, the most important point, to get out of these data.

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analysis of shock, and the other is the realization that ultimately all efforts toward handling shock have got to be directed towards taking care of people. You have made a distinction between 36-hour survivals and animals that lasted totally. For the purpose of analyzing the data, you may want to emphasize that difference For the purpose of planning therapeutic regimes, will it be possible to go on from here and add, perhaps, one or two more things and save these animals?

Fine: For one thing, antibiotic therapy in the recovery period can be continued to see whether the antibiotics, as we have given them, inhibit bacteria sufficiently to allow recovery from shock, but not enough to prevent the activated bacteria from reasserting their pathogenicity, so that they may kill later while the defenses of the dog are still weak Survival beyond 36 hours may be a matter of balance between persisting pathogenicity, the degree of natural defense, and residual antibiotic activity.*

Knisely. It might be possible, from these data, to assist in therapeutic regimes that work, even though you do not understand exactly what you are doing

Fine: There are instances in military, as well as in civilian medicine, of shock states not in the least benefited by blood volume therapy, but which are cured as a result of antibiotic therapy Such observations need to be multiplied. If an antibiotic can reverse otherwise fatal shock, I do not care whether its action is antibacterial or nonantibacterial.

Shorr I think perhaps Dr Haist could add something pertinent to this discussion

Haist We were stimulated by Dr Fine's report at this Conference last year, and felt that if infection, or infective products, were fundamental to the development of irreversibility, it should be possible to show the effectiveness of Aureomycin pretreatment in other species, and also with other shocking procedures which are lethal Therefore, we tried a clamping procedure in the rat

One difference between the rat and the dog is that the rat's tissues do not normally contain a lot of bacteria, especially anaerobic bacteria, whereas the dog's tissues do We attempted to culture bacteria from the anoxic legs of rats, kept anoxic for periods of from 5 hours to 12 hours, and were unsuccessful It seemed to us that the infective factor in the rats shocked by this procedure was not great We tried to determine, in the rat, the effect of Aureomycin pretreatment The rat was given 50 mg Aureomycin HCl per 250

*This work is now in progress in my laboratory

the animal for a while through the distal opening When the animal was stabilized, we gave it some antibiotic through the distal end and then shut it off During the following 36 hours, we kept the animal on fluids intravenously. When his weight and apparent health seemed as good as it was at the start, we put him through hemorrhagic shock, which proceeded to irreversibility and death as in control animals. Swab cultures of the gut were sterile. Even though such sterile cultures don't prove that we got rid of bacteria from the intestine, because we made cultures from swabs instead of from biopsies, we are inclined to believe that invasion from the intestine during the shock state is not an essential feature of the development of irreversibility. If intestinal bacteria are already present in the tissues, they are probably more important. In that case, it is not necessary to superimpose the load that additional invasion from the gut would create. But we can't hold to this conclusion definitively until we repeat these experiments with biopsy instead of swab cultures

Green. There is a clinical impression that Chloromycetin may cause a hypotensive reaction I wonder whether that is one of the things that reduced the apparent effectiveness of Chloromycetin. Could you give us some data as to the variability of survival of your controls at different seasons of the year?

Fine: They are surprisingly uniform throughout the year. The figure is almost the same in every group from one month to the next.

Engel Have you done any experiments on animals treated with cortisone?

Fine. Yes, we reported those a long time ago Cortisone does not do these animals any good, intravenously or otherwise. Neither does ACTH.

Engel. Theoretically, it should do them harm

Fine: Perhaps it did, I don't know. But it did not make any difference in the survival.

Engel. It should increase the rate of invasion by bacteria in rats and mice, which ordinarily do not have bacteria. There is a tremendous invasion of bacteria after cortisone

Cotzias: But these bacteria do not bother the animals, do they?

Engel. Yes, they certainly do Rats given large doses of cortisone for various periods of time are perfectly happy until the point of bacterial invasion, but then they die at a rapid rate, with lung abscesses and all sorts of infections.

Knisely Two major considerations arise in connection with these experiments One is the question of what these things mean in an

analysis of shock, and the other is the realization that ultimately all efforts toward handling shock have got to be directed towards taking care of people You have made a distinction between 36-hour survivals and animals that lasted totally. For the purpose of analyzing the data, you may want to emphasize that difference. For the purpose of planning therapeutic regimes, will it be possible to go on from here and add, perhaps, one or two more things and save these animals?

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gm daily for 6 days by stomach tube, and a priming dose of 100 mg per 250 gm. was administered 15 minutes prior to the clamp release This Aureomycin pretreatment did not appreciably affect survival time in the rats shocked by a clamping procedure If anything, in some of the rats, the survival time was shortened. Nor did it affect the ultimate survival of the rats This was true whether they were clamped for 5-hour periods, or for 12-hour periods.

These results made us feel that one should be careful in interpretation of the results of Aureomycin pretreatment I suggest that it might be possible to distinguish between the metabolic effects of Aureomycin and the antibacterial effects by repeating some of Dr Fine's experiments in the rat

Fine. The fact that the Aureomycin did not do the rat any good might argue, for one thing, that Aureomycin in dogs does not act by exerting a pharmacologic action If Aureomycin succeeds only when shock is due to bacterial action, one would not expect it to do anything for the rat in shock, since there are no bacteria in its tissues

Our data on the bacteria in the rat are the same as Dr. Haist's, in fact, we have gone on, as a result of this observation, to make a study of the normal incidence of bacteria in the tissues of many species The rabbit is intermediate between the dog and the rat. The hamster shows the same data as the rat The guinea pig shows a low incidence of bacteria

The death in Dr Haist's animals has to be explained Why did they die? If they didn't die of bacterial action, what did they die from? He told me last night that he treated these animals with fluid

Haist. Not these particular animals, this is an entirely different series

Fine Then these animals, of course, died of fluid volume deficiency But you did have other animals not treated with Aureomycin, which were treated with fluid volume therapy

Haist Fluid therapy with dextran, or PVP (Polyvinylpyrrolidone) increased the survival of rats shocked by this procedure to 50 per cent, whereas 90 per cent of the untreated rats, without any fluid therapy and with the 5 hour clamping period, died Incidentally, pretreatment with 20 mg Dibenamine 20 hours prior to clamp release dramatically improved survival

Shorr. I think Dr Remington will be pleased to hear that

We are, in this situation, resorting to comparative biology We have a dog whose tissues are infected, protected with antibiotics,

and a rat whose tissues are not infected, unprotected by antibiotics.

Burton. May I ask a question? I wasn't clear whether Dr. Haist was studying the same thing Were you studying irreversibility even when you give transfusion, or were you just studying death due to the shock from clamping? Dr Fine, it seems to me, is studying the effect of these antibiotics upon the irreversibility to transfusion

Fine. Right.

Burton: It seems to me you were not You were not retransfusing these animals at all, were you?

Haist· No, we were not If transfusion was done after a period of hours, it was likely to be ineffective It is necessary to transfuse fairly soon after the time of clamp release in order to have the transfusion effective.

Burton. You didn't see whether the antibiotic had any effect upon the retransfusion recovery?

Haist No, it is quite a different procedure, more drastic, I believe, and that is why I say that in order to determine whether the effect of the Aureomycin in Dr Fine's type of experiment is due to the antibacterial effect, or the pharmacologic effect, his experiment should be repeated in the rat or in some other animal that has tissues relatively free from bacteria I do not claim that this is a repetition of his experiment, but I do feel that if an infective factor is fundamental to irreversibility, then the Aureomycin pretreatment should prevent death, or at least prolong survival, regardless of how the shock was produced

Fine I would disagree with that.

Shorr Do you mean that you would not expect the development of the shock syndrome to take the same course in the rat and the dog, since there are no bacterial flora in the rat's tissues?

Fine The development of the shock syndrome may, up to the time of transfusion, be precisely the same In both instances, there is a fluid volume deficiency The question is whether the rat is going to respond to fluid volume therapy alone If it is, then it is a rat which is simply suffering fluid volume deficiency If it is not going to respond to adequate fluid volume therapy, then the question is, why not? But before asking this, we might determine whether Dr Haist's 50 per cent survival rate does not suggest that his fluid volume therapy was not always adequate to cover the deficit If the deficit is not covered, no amount of Aureomycin will help, whether there are bacteria in the rat's tissues or not

Zweifach: The question remains as to the cause of death in rats subjected to hemorrhage in which a bacterial factor, originating in the gut, would seem to be ruled out

Fine. Is it exactly the same?

Zweifach. I do not think that it is essential for the shock procedure in the rat and in the dog to be identical in every detail. So far as one can ascertain by any of the physiologic or biochemical criteria which have been used to study the syndrome, the sequence of events is basically similar in the rat and in the dog

Fine. Then the question is whether a rat can be a victim of invading bacteria from the gut or the pharynx during the shock phase. That is a question that needs to be studied

Shorr. Perhaps I could rephrase it this way. that due to the absence of bacteria in the tissues in the rat, one should expect no beneficial influence of antibiotics in the rat

Fine. Unless bacterial invasion occurs, say from the gut, and it can be shown that the irreversibility to transfusion can be made reversible by an antibiotic This is, in fact, one of the things on our protocol

EDITOR'S NOTE:

> Dr Fine reports that since the Conference his laboratory has found in some preliminary experiments in rats that Aureomycin given orally a dose of 150 mg per Kg body weight prevents the development of irreversibility in hemorrhagic shock Bacteria-free tissues, therefore, do not exclude the possibility of bacterial invasion during shock from the gut or elsewhere

Shorr. If, however, a bacteria-free animal were actually protected from an otherwise lethal shock by the administration of an antibiotic, wouldn't that suggest that the antibiotic was exercising a biochemical rather than an antibacterial effect?

Fine. I think I should like to have you ask it over again because I am not sure of the conditions you are putting

Shorr We are trying to see if we can reason out the mechanism by which these antibiotics influence the course of shock in the dog There are two possibilities (*a*) as an antibiotic, because the dog's tissues are full of bacteria, and (*b*) as an agent which influences the behavior of tissues, in a pharmacological or biochemical manner, so as to favor recovery This would have a concurrent effect upon the tissues of the organism; it might be similar to, or somewhat different from the action which it exerts upon bacteria

Dr. Baez, would you tell us about some of the experiments on drum shock in the rat, an animal with a bacteria-free liver, and the effect of antibiotics on it?

Baez. A small series of rats was given Aureomycin 100 mg daily, in two doses morning and evening starting four days before the experimental run. In addition, they received a solution of 100 mg. of Aureomycin in tapwater, by stomach tube, 40 to 50 minutes before drumming. After 700 rotations in the drum 87 per cent survived as compared with 37 per cent in the untreated controls.

Shorr: In other words, antibiotics in the rat with a supposedly bacteria-free liver doubled the recovery rate following exposure to drum shock.

Fine. What happened to the intestinal flora?

Engel. Did you have cultures on these particular rats? I am not convinced that everybody's rats are bacteria-free

Shorr. No, we did not have cultures

Fine. Was the last dose of Aureomycin given in solution?

Baez Yes. The other was given in drinking water.

Fine. That only raises the question again whether there is any advantage in possibly inhibiting the invasion of bacteria in the rat during the shock procedure, but we also need further information about this.

Shorr: If your observations, and Dr. Haist's, on the absence of significant bacterial flora in the rat hold for the particular rat strain we used, then protection was apparently conferred by an antibiotic: not by virtue of what it did to the bacterial flora, but by virtue of its effect on body metabolism, if I may put it in that broad way

Engel Did your control rats get something by stomach tube before you drummed them?

Baez They were given tap water.

Stead. I think it would be of interest to see just what happens to intestinal permeability during drumming For example, suppose you gave these rats a large dose of a dye that is usually not absorbed and then you drummed them, would you find that dye through the body, or not?

Nickerson But they would change equally in Dr Haist's experiment as the circulation failed.

Fine. Dr Nelson has data on the effect of drumming on permeability

Nelson It seems to me it is important that Dr. Haist referred to the sterile limb tissue of the body rather than to the gastrointestinal tract, which is not sterile in the rat, as far as I know. This would suggest a gastrointestinal site rather than a muscle site of antibiotic protection in the rat.

Zweifach: The question remains as to the cause of death in rats subjected to hemorrhage in which a bacterial factor, originating in the gut, would seem to be ruled out

Fine. Is it exactly the same?

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Dr Baez, would you tell us about some of the experiments on drum shock in the rat, an animal with a bacteria-free liver, and the effect of antibiotics on it?

Fine. If they have taken back 40 per cent, they are already losing compensation

Shorr That is right, but suppose you made it 50 or 55?

Fine Then they would be irreversible.

Shorr To anything, and as Dr Haist points out, the degree of shock is far more severe.

Haist On the other hand, it is possible to reverse this type of shock by reclamping the limbs rather late in the course of the development of shock

Shorr That is because you interfere with the progressive character of the deficiency As long as fluid continues to sequestrate in that limb, the progressive reduction in blood volume continues.

Remington. Have you followed the blood picture? Isn't the greatest part of the fluid loss accomplished in the first half-hour after the circulation is re-established?

Haist In measurements of the limb swelling in rats clamped for 12 hours, the swelling went up gradually and reached a peak between one and two hours The swelling was complete within two hours so far as our limb volume measurements were concerned Unfortunately, I do not have these data for the rats clamped for five hours

Shorr Did you measure groin swelling?

Haist We measured up to the groin, but that was all If they were reclamped at two hours, a high percentage of the animals survived While some leakage of fluid might still be occurring, normally these rats would die in about three hours and 20 minutes

Shorr I am constantly struck with the fact that at a precarious level of the circulation a relatively small amount of additional fluid loss is critical

Fine That is right

Nickerson There is still the possibility of material continuing to pass into the general circulation from tissue which must be essentially dead after twelve hours of clamping This is certainly a continuing stress.

Stead I should like to ask whether the animals change any in the way they breathe Were they anesthetized or not?

Haist These animals were not anesthetized

Stead I have always been interested in what happens in the general reactivity of the animals A dog with a tourniquet on moves around and is quite irritable, but immediately after the tourniquet is taken off, its respiration slumps and it is quite a different animal I have never reclamped these animals, but I would assume that

Shorr We subjected our resistant rats to the same degree of hemorrhagic hypotension and presumably the same type of intestinal anoxia as our control rats (71) Why should they have recovered when exposed to the same type of intestinal bacterial flora?

Burton. Do you know that they have the same type of intestinal anoxia?

Shorr Yes, we reduced the blood pressure to 35 mm Hg and maintained it there for two hours We assume they were hypoxic because they produced ferritin, and the blood vessels that are concerned with the liver are also the ones which provide the flow to the gut

Burton. I thought Dr Zweifach this morning was telling us that when he watched the mesenteric flow, it was quite different in these resistant rats from the others

Shorr. I believe that he was describing Dibenzyline-treated rats

Burton I thought it was also said about the resistant rats

Shorr Yes, following rotation in the drum the mesenteric circulation of resistant rats is better maintained than in untrained rats However, what I was referring to here was an experiment in which a rat which had been made resistant to drum shock was subjected at first to moderate hemorrhagic hypotension and then to a period of two hours at 35 mm Hg That is a situation, I believe, which makes for an impairment of the integrity of the mucosa and should favor bacterial entrance

Fine I should think that you might repeat that drum shock experiment with inactivated Aureomycin

Shorr I think that is an excellent suggestion All I mean to indicate is that the possibilities are still very real that we may have a specific tissue reaction rather than an antibacterial one

Fine Haist's experiments wouldn't support that idea, would they?

Haist One difference between our procedure and yours is that ours is more drastic, and yours can be more readily graded

Shorr. That is right

Would you feel that if the uptake were continued for a couple of hours longer, a far lower survival rate would be obtained?

Fine The blood pressure can't be pushed below thirty The dogs won't take it

Shorr Or, if you continued it for two more hours, that is, if you changed the conditions of your experiment and had a per cent uptake of 50 or 55

kept the pressure there for five hours, unless the animal succumbed sooner. At hour intervals we sampled the arterial blood for toxin assay, which was done by injecting 1 ml of dog blood into the peritoneal cavity of mice. As you know, mice are particularly sensitive to botulinus toxin, while dogs are relatively resistant to the toxin Another reason that led us to choose this particular toxin for the tracer was the fact that it has a large molecular weight, about 900,000

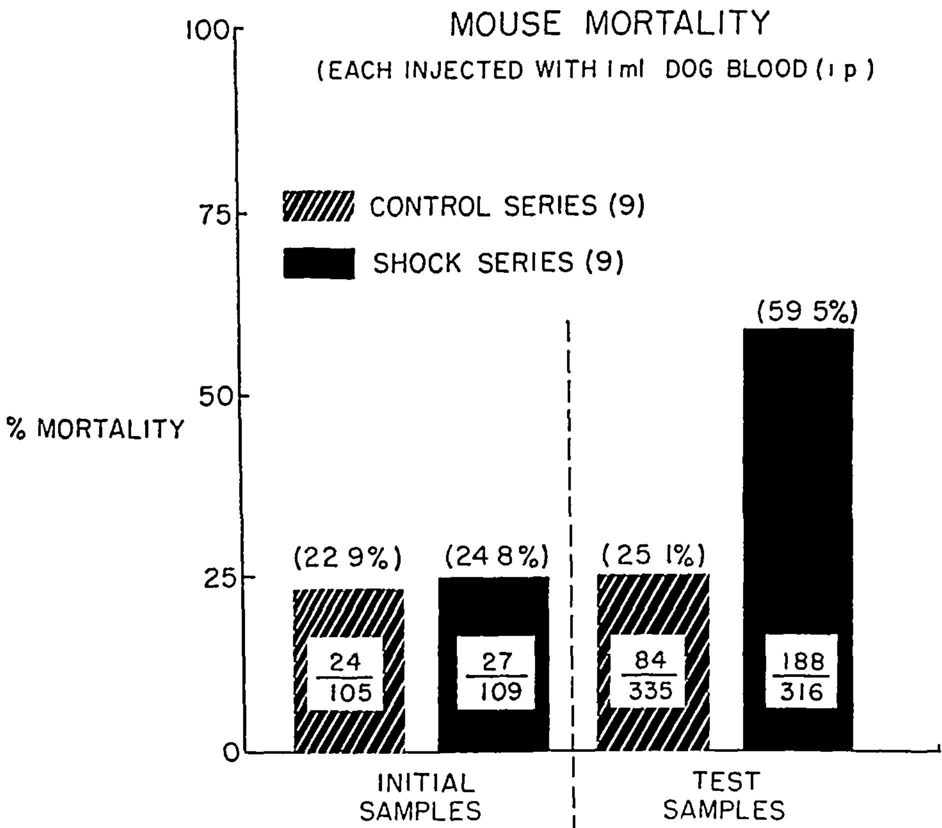


FIGURE 22 The bars to the left of the dotted line represent the number of mice dying as a result of injection of initial blood samples from the control and shock series of dogs The two bars on the right represent the aggregate number of mice dying as a result of injection of blood samples obtained during the 5-hour period of observation in the two groups of dogs Fraction expressed is the number of mice dead over the total number injected Mortality of control mice protected with botulinus antitoxin was 0% Printed, by permission, from Nelson, R M , and Noyes, H E Permeability of the intestine to bacterial toxins in hemorrhagic shock *Surgical Forum* Clin Cong Amer Coll Surgeons 1952 Philadelphia, W B Saunders Co 1953 (p 474)

Figure 22 shows the composite results of the mouse mortality, each injected with one ml of dog blood. The mortality rate with blood from the control dogs was, first of all, 24 dead out of 105 mice injected, or 22 9 per cent, with blood from dogs later to be

the arterial oxygen concentration might drop quite low during this period of lethargy. Do these animals come back up in their breathing when they are reclamped?

Haist: Dogs with cuffs left on for six hours, and then removed, showed a fall in blood pressure which continued along for some time at a low level without too sharp a progressive drop. If at that time narrow band tourniquets were reapplied high on the legs, there was a rather rapid restoration of blood pressure The tongue, formerly cyanosed, became pink rather rapidly, too

Stead: Do they breathe differently and act differently? In our experience, before the tourniquet is taken off, the animal is moaning and groaning As the tourniquet is released, the dog becomes quiet and its breathing changes. I wonder whether, associated with reapplication, stimuli through the nervous system affected its breathing, color, and general level of activity

Haist The fact that the reclamping or reapplication of a tourniquet is a strong stimulus, is probably of importance and may be one of the factors involved in the restoration of blood pressure.

Shorr Am I wrong in believing that this is the experiment which led Cannon to postulate a toxic substance in shock?

Haist In essence, you are right

Shorr Dr Nelson, do you have something to contribute to this?

Nelson: Talking about toxic substances in shock is apt to lead one into a precarious position Dr Stead alluded to something which captured our interest about two and a half years ago when we observed that gram-negative bacteria produced shock and death in experimental animals, giving a rather characteristic metabolic pattern, the same as Dr Engel pointed out this morning In the last year, we have learned only too painfully that the same thing occurs in humans if they get gram-negative bacteria in blood transfusions (72). With the knowledge that gram-negative bacterial products, presumably the endotoxins, have a deleterious effect on the capillaries, we were anxious to know whether or not in shock there was any difference in concentration of a bacterial toxin which was originally present in the intestine We performed an experiment using botulinus toxin as the tracer material, putting it into the stomach of a dog by gavage and allowing two-and-a-half to three hours for the mixture to enter the distal small bowel and colon. Barium sulfate was added to the toxin so that localization of the mixture could be documented by roentgenograms We then subjected the dog to a sterile hemorrhagic shock procedure somewhat similar to Dr. Fine's (73). We bled the animals to 40 mm. Hg and

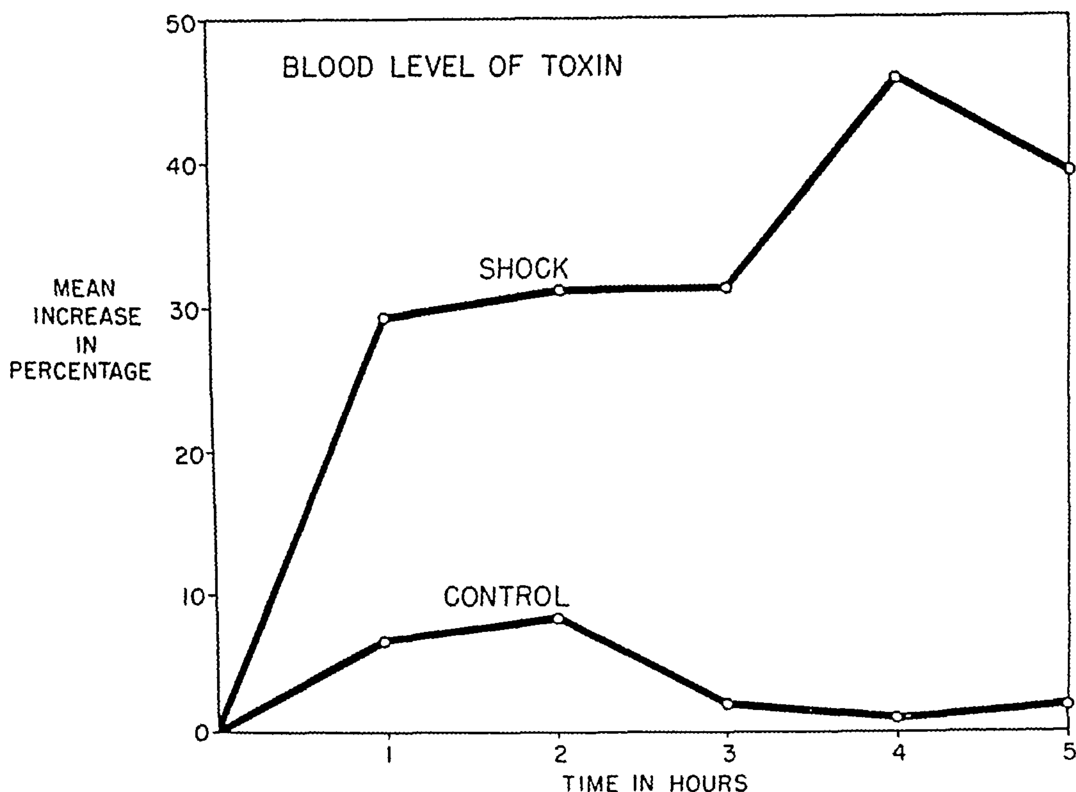


FIGURE 24 This graph represents the mean results in mouse mortality for the two groups on the basis of hourly samples The ordinate is an expression of the mean increase in the per cent of mice dying from the intraperitoneal injections of dog blood Printed, by permission, from Nelson, R M, and Noyes, H E Permeability of the intestine to bacterial toxins in hemorrhagic shock *Surgical Forum* Clin Cong Amer Coll Surgeons, 1952 Philadelphia, W B Saunders Co 1953 (p 474)

Figure 24 shows on an hourly basis, the mean increase of mice dying as the result of the injection of dog blood I am sorry the standard error is not shown The one-hour difference shows an overlap on the standard error, but at the two- to five-hour levels, there is no overlap on the range Thus, we know that this is a statistically significant increase in mouse mortality. We know that the death of these mice was due to botulism because for each sample of dog blood there were several mice, three to five in each group, protected with botulinus antitoxin, and of that group of 389 that were protected with antitoxin, there was only one death

Blood culture results from the initial and final blood samples as tabulated above The final control samples are from the jugular vein, all others from the femoral artery

Table VI is of interest because we took blood cultures before and after, just to correlate, if we could, any increase in bacteria while we were getting this increase in toxin It shows that we found no significant difference in peripheral blood cultures for bacteria in

bled, there was a similar mortality To begin with, blood levels of toxin were comparable. The aggregates for the one- to five-hour analyses are tabulated on the right side of the figure In the control group of dogs, the mortality rate was statistically similar, being 25 1 per cent of 335 mice injected, whereas blood taken from animals in shock produced a mortality rate of 59 5 per cent

Another way of analyzing the data, rather than by mortality, is the actual level of toxin in the blood as expressed in mouse MLD (minimum lethal dose) units We analyzed the data in this manner, comparing mortality rates with the mortality of mice given injections of known concentrations of toxin That is summarized in Figure 23, showing the same four groupings

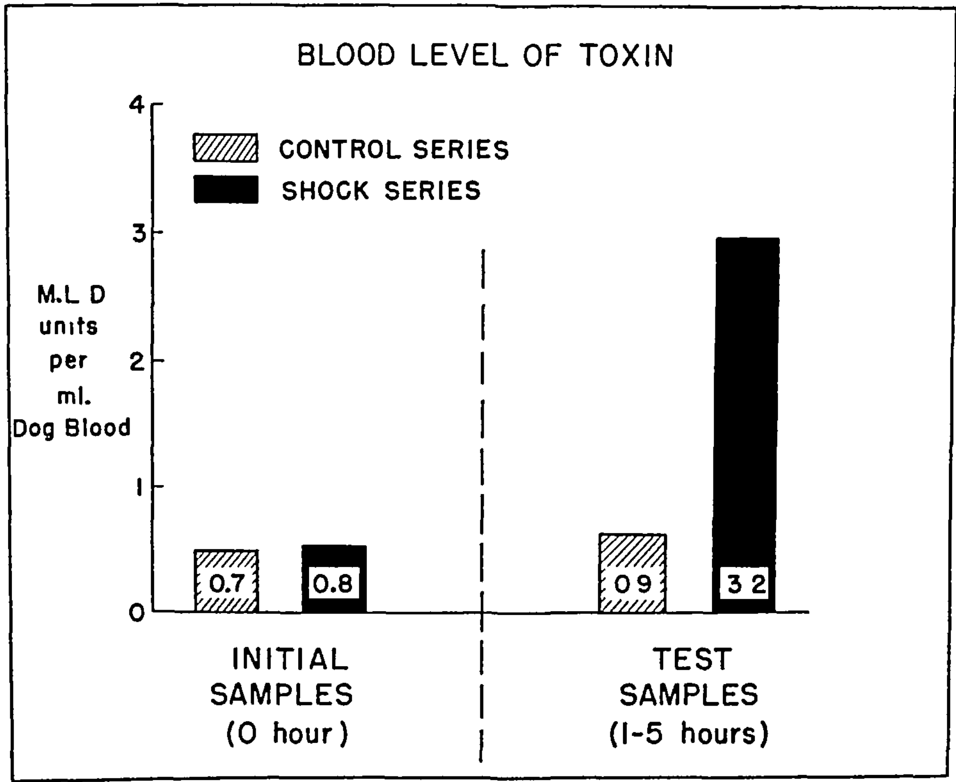


FIGURE 23 Concentration of toxin in dog blood as expressed in mouse MLD units is plotted for the 2 groups of dogs before and during the shock period

In the initial samples taken three hours after this gavage, the two groups of animals had 0 7 to 0 8 MLD botulinus toxin in their blood. In the central group the level average was 0.9 MLD, but in the shock group it was 3 2 MLD during the ensuing 1 to 5 hours after hemorrhage

the two groups. We had a slightly higher incidence of positive cultures in the control dogs in the final analysis. I might mention that this sample was taken by a percutaneous jugular puncture, which probably explains the difference

There are only two conclusions which I would wish to draw from these data. One is the fact that a blood culture of bacteria is not a measure of the effect of bacterial organisms. It merely reveals the presence of living bacterial cells and not the effect of the products of bacteria, which apparently are the deleterious agents in infection. The other conclusion is that a large molecular weight bacterial toxin, initially present in the intestine of the dog, is present in higher concentration in the blood of dogs in shock than in control dogs not in shock Whether this increase in toxin is due to increased gut permeability, or whether it is due to decreased detoxification or decreased secretion by the kidneys, we cannot infer from these data The important thing is that there is an increase, and the mechanism of its increase is of secondary but tremendous importance

Obviously, the thing we should like to know is not the effect with botulinus toxin but with gram-negative toxins, which are certainly toxic to capillaries At the time we did these experiments, we didn't feel that the techniques for measuring these toxins were precise or sensitive, however, we are working to that end and hope that we shall be able to answer the question specifically as to whether or not gram-negative bacterial toxins achieve concentrations significant enough to make any difference in animals, or in humans, in shock

Shorr. Have you done any similar studies with animals pretreated with Dibenamine?

Nelson No, but along that vein, Penner (74) has reported that pretreatment with adequate doses of ergotamine tartrate or tetraethylammonium chloride prevented the appearance of the changes in the intestines ordinarily produced by the intravenous infusion of B Shiga (*Shigellae dysenteriae*) toxin into dogs That is the only lead I have in answer to that question It certainly would be interesting to try

MECHANISM BY WHICH BACTERIA EXERT A DELETERIOUS EFFECT ON THE CIRCULATION

Zweifach In discussing the contributory role of infection in shock, what evidence is there concerning the mechanism by which

TABLE VI

Blood Cultures

CONTROL DOGS		Pair No	SHOCK DOGS	
Initial	Final		Initial	Final
Clostridium perfringens	*Cl perfringens*	1	Sterile	Sterile
Sterile	*Escherichia coli* and *Cl perfringens*	2	Sterile	*Cl perfringens*
Bacillus subtilis	*Non-hemolytic streptococcus*	3	Sterile	Sterile
Diphtheroids	Diphtheroids and *Beta-hemolytic streptococcus*	4	Sterile	Sterile
Sterile	Sterile	5	Sterile	Sterile
Sterile	Sterile	6	Sterile	Sterile
Sterile	Sterile	7	Sterile	Diphtheroids and *Micrococcus pyogenes var albus*
Sterile	Sterile	8	Sterile	Sterile
Sterile	Sterile	9	Sterile	*E coli*
Sterile	Diphtheroids	10	Sterile	Sterile
Sterile	Sterile	11	Sterile	Sterile
Sterile	Diphtheroids	12	Sterile	Sterile

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Zweifach In discussing the contributory role of infection in shock, what evidence is there concerning the mechanism by which

bacterial toxins per se exert a deleterious effect on the circulatory hemodynamics during shock?

Fine: Burrows' review, *Endotoxins* contains a number of different purified extracts and bacterial antitoxins, which are described as having a vascular toxic action (75,76).

Nelson: The conclusion is that gram-negative bacteria toxins exert their toxic action by virtue of their effect on the capillaries.

Zweifach: I am not questioning the thesis that bacterial toxins exert a deleterious effect on the peripheral blood vessels, especially the capillaries My question was directed at the relation of these agents to the particular type of vascular dysfunction observed during the shock syndrome Most references indicate that bacterial toxins produce capillary dilation and increased capillary permeability I am not certain that such effects have been shown to occur in the terminal vascular bed of any organ during hemorrhagic shock

Nelson. That is correct

Zweifach. Is there any evidence of a change in capillary permeability in irreversible hemorrhagic shock?

Nelson I don't know that it changes capillary permeability except that the diameter of the capillaries is increased The hematocrit rises Presumably, there has been an extravasation of fluid in the interstices

Fine. I don't know that Burrows said that there was an increase in capillary permeability The various things that different toxins can do include vascular damage, which is a property common to all of them I may be mistaken, but I don't think he describes their action on the vascular tree precisely in terms of capillary permeability

Nelson He uses vascular poison rather than permeability

Zweifach My point was raised in order to differentiate between the direct vascular effects of bacterial toxins and those attributable to a wide variety of indirect metabolic influences which such toxins may have What is the relative importance of infection in influencing the course of different types of clinical shock?

Stead. Infection would rate extremely high

Fine. Dr Churchill, referring to his military experience, said there was a distinction between the results of fluid volume therapy in wound shock and in hemorrhagic shock In the latter, the patient got better with transfusion even if he had been hypotensive for as long as 14 hours, whereas patients in wound shock did not always show this response Too many of them died after they had appro-

priate fluid volume therapy So here is a distinction which is valid. The inference that these patients who did not survive were septic was not made by Dr Churchill. But they all had wounds, since the condition is called "wound shock" I raise the question whether these men can be said not to have been septic simply because there were no overt signs of sepsis These wounded men might have been succumbing to bacterial products without being able to produce constitutional or even local signs of sepsis

Zweifach. Aside from the usage of the terms "capillary dilation" and "increased capillary permeability" — gross descriptive terms at best — the hemodynamic implications of infection apparently are not very well understood

Stead. I think all that can be said is that infection will produce failure of the circulation, but the exact mechanism by which it does so, as far as I know, is unknown

Fremont-Smith. In typhoid vaccine injections in man, I have seen the blood pressure go down to shock levels in the chill stage Also in malaria, with the onset of acute infection, there is a condition which clinically cannot be distinguished from shock except that in addition to shock the patients have fever

Burch· There are a number of clinical examples which are labeled "shock-like" syndromes, such as the Waterhouse-Friderichsen syndrome, in which there are metabolic disturbances and a decline in blood pressure B botulinus infection with so-called "ptomaine poisoning" is another example

Fremont-Smith Fulminating flu

Burch Surely Also acute meningitis

Zweifach What are the circulatory changes leading to the development of a state of shock in these patients? Are they the same as those observed following hemorrhage?

Burch· I do not know There is a rapid, thready pulse, a decline in blood and pulse pressure, sweating, and so forth

Zweifach It is, of course, possible to produce a state of shock by bacterial toxins alone Freedberg, *et al* (77), several years ago studied the circulatory changes during shock produced by Shiga endotoxin I reported on observations in the omentum following the administration of several different Clostridia toxins A state of hypotension and circulatory collapse developed after several hours, secondary to a diffuse change in vascular permeability and a loss of fluid from the circulatory tree into the tissues Prior to these changes, no alterations in the functional status of the capillary bed could be detected The sequence of vascular changes which

ensued, after the vascular system started to lose fluid, was similar in its course to other types of circulatory failure which had been studied.

Fine: These toxins exhibit multiple effects. Von Heyningen describes three or four properties of these toxins Some increase vascular permeability, and some do not. In the face of a multiplicity of deleterious effects, the organism does not respond to fluid volume therapy alone. We have seen hypotensive patients with septic peritonitis who for all the world look like patients who have simply lost a lot of blood, but they come right out into a pink, dry state, with normal blood pressure after antibiotic therapy, without transfusion

von Euler. It is well known that some smooth muscle is extremely sensitive to toxins of various kinds I think every pharmacologist has noted that For instance, a piece of rabbit's intestine taken out half an hour or so after the death of the animal hardly responds to anything It may very well happen that an isolated piece of fowl's rectal caecum does not respond at all to epinephrine or nor-epinephrine, if the tubing supplying the bath solution is not thoroughly cleaned every day If smooth muscle, in general, is extremely sensitive to toxins, that may very well be the case for the vessels, too. They might not respond to the normal vasoactive substance

Knisely. I think Dr von Euler has touched on a considerable vacuum in our knowledge we know that infection is bad for people, we know that often they will die, but the biochemistry of the mechanism of that death is quite unknown to us

Burton. May I put in a plea, in our obvious ignorance about the hemodynamics and changes in permeability, particularly in the human, for making use of all the new methods of investigation as they come out? It seems to me that a most attractive one, which is easily done, is the clearance of radioactive sodium, or other radioactive ions or substances, by injecting some smooth tissues in order to see how quickly it disappears It is true that it is being used to measure blood flow, but obviously the rate at which it disappears is a combination of the flow and the permeability properties It would seem to me that in this problem of shock, particularly in humans, we might learn a great deal by applying that method

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DISTRIBUTION AND POSSIBLE PHYSIOLOGIC FUNCTIONS OF EPINEPHRINE AND NOR-EPINEPHRINE

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THE WORK I am going to speak about has not been concerned, to a very great degree, directly with the problems of shock However, some of the things I shall report on may have an indirect bearing on that problem

We* have been interested in the distribution of the sympathomimetic amines nor-epinephrine and epinephrine in the body tissues and organs, their formation during various conditions, and the function and differentiation between the different kinds of these sympathomimetic substances To some extent, we have also had our interest directed to their actions, but I know there will be more competent reviewers of that particular field.

DISTRIBUTION OF EPINEPHRINE AND NOR-EPINEPHRINE IN VARIOUS TISSUES

Table VII gives an idea of the amounts of the two chief sympathomimetic amines which appear in different tissues (1) The spleen is rather rich in nor-epinephrine, whereas in the other organs the amounts are smaller In the heart, for instance, it is only about one-third of that in the spleen In the liver, it is still less, and in an organ such as the striated muscle, very low figures are found

From the last column of Table VII, it can be seen that the percentage of epinephrine in terms of total catechols, that is to say the sum of nor-epinephrine and epinephrine, varies quite a lot In the spleen, it is generally low, ranging from zero, or nearly

*I wish to extend my grateful appreciation to Dr E Ostlund, Mrs A Purkhold, Miss S Hellner, Mrs U Hamberg, and Mrs I Orwén, whose skilful assistance has contributed largely to many of the results referred to in this presentation For financial aid, I am indebted to the National Medical Research Council of Sweden, the Rockefeller Foundation, and the Astra Ltd , Södertalje

TABLE VII

ORGAN (sheep)	Nor-epinephrine	Epinephrine	Epinephrine %
	µg./gram	µg./gram	
Spleen . .	3.0-3.3	0-0.11	0-3.4
Parotid gland .	0.5-2.2	0.03-0.19	5-14
Submaxillary gland	0.4-1.1	0.10-0.21	12-21
Heart . .	0.6-1.1	0.1-0.2	10-20
Kidney . . .	0.4-0.6	0.05-0.07	11
Liver . . .	0.15-0.20	0.007-0.011	4-7
Lung	0.08-0.1	0.002-0.01	2.5-10
Striated muscle .	0.025	0.0013	5

zero, up to something around three or four per cent. In other organs, such as the heart, the relative amount of epinephrine is rather higher, at least in some animals. That has been found not only in our laboratory by Dr. Goodall (2), but it has also been confirmed by others (3), and I think it is probably a finding of some significance. In the parotid and the submaxillary gland, the epinephrine figures are relatively high.

There was one organ in which no nor-epinephrine was found at all, and no epinephrine either, and that was the placenta. Since it is well-known that the placenta completely lacks nerves, the inference was that the presence of nerves had something to do with the amount of the sympathomimetic amines. That led us to look at the contents of the nerve trunks themselves. Work of this kind had been done earlier by Cannon and Lissák (4), who found what they thought was epinephrine in sympathetic nerves.

Table VIII shows the content of *l*-nor-epinephrine in various nerve structures (1). The brain* and spinal cord, contain relatively small amounts of nor-epinephrine. The same is the case for several nerve trunks, such as the vagus, the phrenic, the saphenous, the cervical sympathetic, and the superior cervical ganglion. In the other nerve trunks, which contain a relatively high amount of postganglionic sympathetic nerves of the adrenergic type, the amount is higher. This is especially true for the splenic nerves where the nerve trunk, freed from its sheath, may contain as much

*We have confirmed the recent finding of M. Vogt that the hypothalamic region is rich in nor-epinephrine.

TABLE VIII

Amount of *l*-Nor-epinephrine in μg. per Gram of Nerve

Nerve	Cow
Brain	0 04-0 2
Spinal cord . . .	0.12
Vagus	0 1
Phrenic	0 15-0 25
Saphenous . .	0 2-1
Cervical sympathetic . .	0 6
Superior cervical ganglion .	1
Mesenteric . .	1 5-3
Sympathetic trunk .	2 5-4 9
Splanchnic . . .	4
Splenic	8 5-18 5

as 18 5 μg per gm of fresh nerve This seems to indicate that there is a correlation between the number of adrenergic fibers in the nerve trunk, and its content of nor-epinephrine There is no column included in Table VIII for the amount of epinephrine in these nerve structures, but generally it can be said that the amount is low and seldom exceeds 5 or 10 per cent of the total sympathomimetic amines

Loewi. What kind of nerve is the splenic nerve?

von Euler The splenic nerve is histologically composed of unmyelinated fibers, and it gives an almost pure C-fiber electrogram. Some of these fibers, undoubtedly, are afferent, but the majority of them are postganglionic sympathetic ones

Loewi That might be the reason you found so much nor-epinephrine What else could explain it?

von Euler. The splenic nerve has a larger proportion of adrenergic fibers than the other nerves listed, and we think that the adrenergic neuron itself produces nor-epinephrine by some enzymatic action. Since nor-epinephrine is present not only in the nerve trunk, but also in similar amounts in ganglia, it seems that the whole neuron is capable of forming nor-epinephrine

Nickerson Do you have any thoughts about the possible concentration of these sympathomimetics in the nerve endings or in the effector cells? The ratio of concentration in the nerve to concentration in the whole tissues is on the order of 2 or 3 to a maximum of 6 to 1, and yet the ratio of nerve tissue to other tissue in these end organs is probably several hundred to one. There must be some sort of concentration. Have you thought about where this concentration may be?

von Euler. Taking the content in the splenic nerve as 15 µg per gm , and in the spleen as 3 µg. per gm., gives a ratio of 1 to 5. Obviously, the spleen does not contain sympathomimetic nerve fibers in the proportion of 1 to 5, which necessarily means that the concentration in some part of the intrasplenic nerves, if the substance is exclusively bound to the postganglionic sympathetic fibers, must be much higher than in the nerve trunk.

Engel· Are these determinations by chemical analysis, or by biological assay?

von Euler. They are made in the following way· the nerves, and the same is also the case for the tissues, are ground and extracted with trichloracetic acid, about 5 per cent, or acid alcohol: it does not make much difference To the trichloracetic acid filtrate is added aluminum sulfate, to about 0 1 per cent After this, sodium hydroxide is added to 7 6, and the ensuing precipitate is filtered off The precipitate then contains all of the sympathomimetic amines of catechol nature The precipitate is dissolved in normal sulfuric acid, and the aluminum salt is precipitated by the addition of four volumes of a mixture of acetone and alcohol. After filtration, the acetone and alcohol are driven off and the aqueous solution is tested biologically Usually the amounts obtained from tissues are so small that they will not permit a colorimetric estimation, but they generally allow a biological assay

Zweifach Could you describe this physiologic test?

von Euler To differentiate nor-epinephrine and epinephrine in a mixture, the extract is assayed on two test preparations. one of them having properties such that nor-epinephrine is more active, although acting principally in the same way as epinephrine, and in the second preparation, epinephrine should be the more active By estimating the activity ratio of epinephrine to nor-epinephrine in both cases, and by estimating the activity of the extract in terms of one or the other, one can compute the results and get the answer in terms of nor-epinephrine and epinephrine

Shorr: Would you introduce into the record the two systems that you use?

von Euler We have been using the cat's blood pressure and the fowl's rectal cecum In the cat's blood pressure, nor-epinephrine is about 2 to 5 times more active than epinephrine, giving an activity ratio of 0.5 to 0 2, whereas on the fowl's rectal cecum, epinephrine is something like 20 to 60 times as active as nor-epinephrine, giving an activity ratio of 20 to 60

Ahlquist It has been shown recently by Swanson and Chen (5) that in cats pithed by their method, the ratio between the activity of epinephrine and nor-epinephrine is just the reverse of what you state; that is, epinephrine is much more potent than nor-epinephrine in increasing blood pressure Therefore, if you had a cat which did not follow the general rule that nor-epinephrine is the more potent pressor agent, would it make any difference in your biological comparison?

von Euler We are using the cat under Nembutal anesthesia, and in order to increase the sensitivity of the preparation, we always use a priming dose of ergotamine in the amount of about 0 15 mg per Kg, which stabilizes the blood pressure and increases the response to epinephrine and nor-epinephrine to quite a good degree (1)

EDITOR'S NOTE Dr von Euler wishes to add this Conference "afterthought:"

> It would reduce the differentiating ability of the system, which depends on the relation between the activity ratios With an activity ratio of 30 on the fowl's rectal cecum, and 0 3 on the cat's blood pressure, the relation is $\frac{30}{0\,3} = 100$ If the denominator is raised, as in the case suggested by Dr Ahlquist, the relation may be only $\frac{30}{3} = 10$ If the relation is 1, there is obviously no means of differentiation between nor-epinephrine and epinephrine

For the present discussion it might be of special interest to see whether these substances are present in the vessels This has been studied in some detail by Schmiterlow (6) The upper curve in Figure 25 represents a blood pressure tracing of the cat, and the lower curve the movements of the uterus in situ As Barger and Dale (7) have shown, the virgin uterus of the cat relaxes much more markedly in response to epinephrine than it does to nor-epinephrine It is obvious from Figure 25, Section 1, that extracts of the mesenteric artery, and the portal vein, have a much smaller effect on the uterus in situ than does epinephrine in a

dose which gives a comparable effect on the blood pressure. This experiment is of a similar kind to those made by Cannon and Rosenblueth (8). They also used this difference in response to sympathin liberated from vessels, and epinephrine, although the same inferences were not drawn

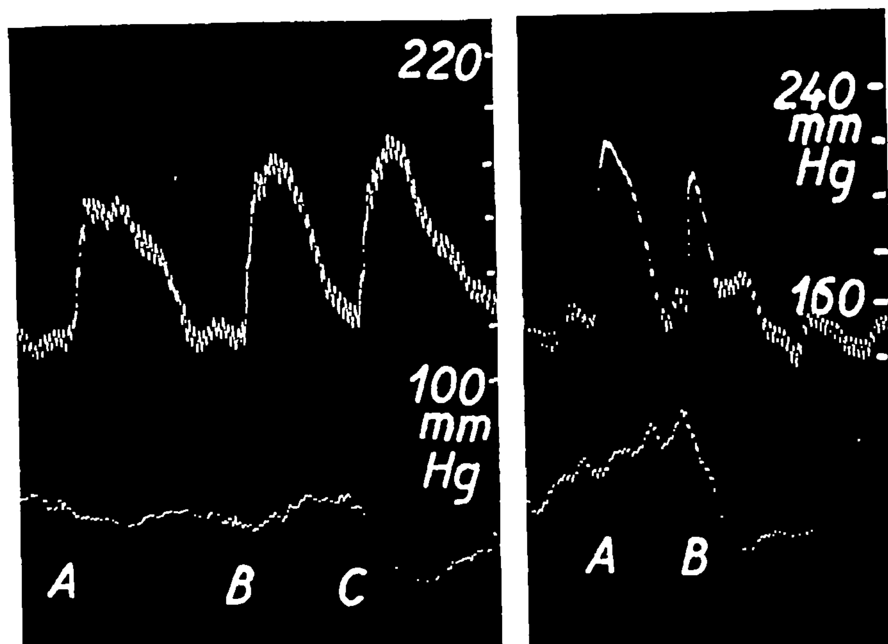


FIGURE 25 Cat, chloralose, adrenals removed Upper curve, blood pressure, lower curve, movements of non-pregnant uterus in situ (relaxation downward) Animal treated with atropine and antergan

I A Extract of 1 g horse mesenteric arteries
B Extract of 1 g horse portal vein
C 3 μg epinephrine

II A Extract of 1 g horse coronary vessels
B 2 μg epinephrine

Reprinted, by permission, from Schmiterlow, C G The nature and occurrence of pressor and depressor substances in extracts from blood vessels *Acta physiol scandinav* **16**, *Suppl 56 (1948)*

Figure 25, Section 2 shows the effect of an extract of coronary vessels, which is of some interest At A, one can see that the effect of the extract of the coronary vessels is strong on the blood pressure but practically absent in the uterus *in situ*, whereas epinephrine gives a strong relaxation The active substance in the coronary vessels thus cannot possibly be epinephrine, but agrees in action with nor-epinephrine

Green Have you tried a mixture of epinephrine, and nor-epinephrine, on the uterus to see if the nor-epinephrine might inhibit the relaxation due to the epinephrine?

Shorr. Would you introduce into the record the two systems that you use?

von Euler. We have been using the cat's blood pressure and the fowl's rectal cecum. In the cat's blood pressure, nor-epinephrine is about 2 to 5 times more active than epinephrine, giving an activity ratio of 0.5 to 0.2, whereas on the fowl's rectal cecum, epinephrine is something like 20 to 60 times as active as nor-epinephrine, giving an activity ratio of 20 to 60.

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seems rather high, but since it involves a chemical preparation, and two different test preparations, it is probably the best that can be done unless we had a very large series

Nickerson: I was thinking particularly of the variability in different animals Can you consistently reproduce the 0 4 µg. figure three or four weeks after denervation?

von Euler That would depend on the degree of regeneration, which is unpredictable However, one would never find a figure as high as 0 4 µg. per gm in the freshly denervated heart. In the normal heart, I believe, the limits were around 0.5 and 1.

Knisely. If a piece of tissue were cut into, let us say, six parts, and one sample were prepared for testing immediately, while the others were tested in an hour, or at various later times, what would be the nature of the decrease in the active substance with time?

von Euler. The decrease is quite slow We have not been able to detect a significant difference in the content, for instance, of the spleen when one piece is processed at once and the rest is left at room temperature for twelve hours.

Burton. Does the pH make a great deal of difference?

von Euler. The organ is left as such, and then extracted in the usual way in an acid medium If, on the other hand, the organ is deep-frozen, and then thawed and left at room temperature for as short a time as one hour, practically all activity disappears Therefore, one cannot use frozen specimens unless they are processed directly

Engel Is this true of the epinephrine content of tissues, as well as nor-epinephrine?

von Euler. This is true for both.

Engel It holds for quite a series of organs?

von Euler. We have had experience with a number of organs, and it has been the same

Loewi. If one heart were left intact, and another mashed, and the determination were made after two hours, there should be a difference in the effects obtained

von Euler. There would be a considerable difference, yes The active substance would remain only in the intact organs

Moe With respect to Dr Burton's remark about the pH, epinephrine is much more stable in blood than in saline at the same pH

Burton. I was thinking about the activity of the enzymes which destroy these amines. It is very much affected by pH. However, I see that my question was irrelevant in connection with leaving the tissue as it is

von Euler: Yes, it has been done, not only in this kind of experiment, but also, of course, to check the method of differentiating the two in an extract

If the presence of sympathomimetic amines in the organs is due to the sympathetic nerves, it should be possible to remove that action by letting the nerves degenerate Such experiments were made by Cannon and Lissák (4), and they found that the content of the tissues in such substances decreased to a great degree.

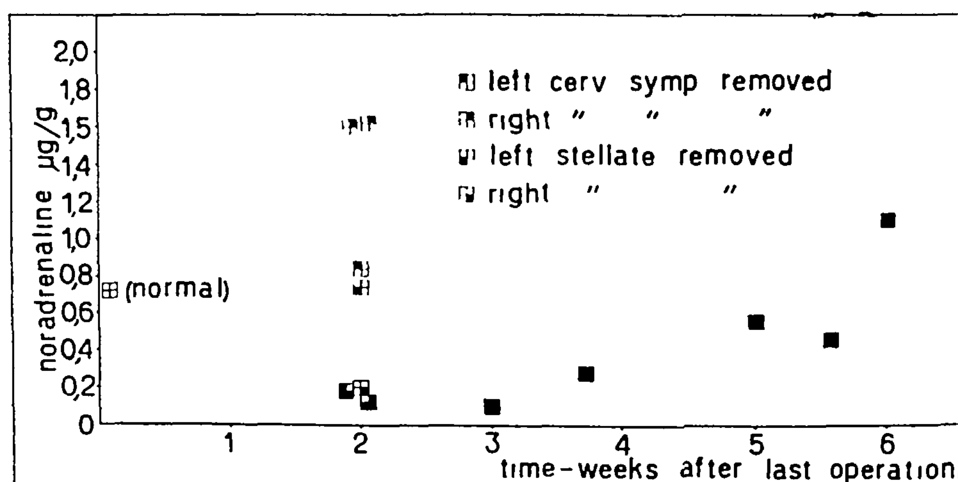


FIGURE 26 Graph indicating the nor-epinephrine titer in sheep heart after various operative stages and various times after complete cervico-stellate ganglion ectomy Reprinted, by permission, from Goodall, M Studies of adrenalin and noradrenalin in heart and suprarenals *Acta physiol, scandinav* **24**, *Suppl 85 (1951)*

Figure 26 is taken from a paper by Goodall (2), who worked in our laboratory on the effect of the innervation on the presence of sympathomimetic amines in the heart of the sheep. After denervation of the heart, the content went down quite considerably It was obvious that removal of the right stellate was most effective in denervating the organ The content remained low for a time, but after some two or three weeks a gradual increase was noted, and after some six weeks the content was about as high as it was in the beginning. Histologic examination showed an ingrowth of postganglionic fibers from the sympathetic trunk Therefore, the conclusion from these experiments was that the presence of the sympathomimetic amines depends on the presence of the adrenergic nerves

Nickerson. Dr. von Euler, can you give us an approximate error for these figures?

von Euler. With the biologic method, the standard error is between 10 and 30 per cent, depending on the conditions That

TABLE IX

Epinephrine and Nor-epinephrine Content in Suprarenal Glands, From Various Animals

Animal	Nor-epinephrine mg./gm.	Epinephrine mg./gm.	% Epinephrine
Baboon	0	0.83	100
Rabbit	0-0.2	0.4	94-100
Guinea-pig	0.01-0.07	0.5	88-98
Rat	0.16	0.9	84
Man	0.05-0.1	0.3-0.8	80-90
Cow	0.5-1.3	1.8-3.3	75-85
Zebra	0.1-0.3	1.4-1.9	70-85
Sheep	0.4-0.9	0.8-1.5	55-80
Dog	0.5	1.6	76
Impala	0.2	0.6	75
Grant Gazelle	0.3-0.4	0.6-0.7	65
Thompson Gazelle	0.36	0.56	61
Wildebeest	0.6	0.83	57
Squirrel	0.1	0.1	50
Cat	0.4-0.8	0.4-0.8	40-60
Toad	1.2-2.4	1.2-2.7	40-60
Lion	0.3	0.2	40
Dogfish	6.0	3.0	30
Whale	0.5-2.5	0-1	0-50

von Euler: Small amounts of epinephrine are present in chromaffin cells outside the suprarenals.

Shorr: Is it the same for the dogfish? My recollection is that in the dogfish the adrenal cortex is an entirely separate structure from the adrenal medulla. It was because of this separation that it was first possible for Biedl (13) to show clearly that it is the adrenal cortex rather than the medulla which is essential for survival.

von Euler. In the dogfish, the preparation taken was the so-called "ganglia," which appear like a string of pearls at the back of the

von Euler We were afraid of that at first, and were surprised that the organ could be left as it is at room temperature for many hours without any destruction of the catechol amines Apparently, the destroying enzymes have no access to the active substance.

Engel. Do you think this would hold true for room temperatures in America, which are probably a good deal higher than in Europe?

von Euler I do not know

Cotzias· The same experience has been reported by Dr Barsoum of Cairo, Egypt, in connection with histamine (9).

von Euler· I am very interested to hear that.

Shorr. At that temperature your tissues, except for the surface, are really anaerobic, and the pH would shift toward the acid side. Both circumstances might be favorable for survival

von Euler. That is true So much for the presence of catechol amines in organs and nerves Nor-epinephrine has been shown to be present also in the suprarenals, as demonstrated first by Holtz, and his collaborators (10), independent of our experiments on the tissues although published later

Table IX shows that the relative and absolute amounts of epinephrine and nor-epinephrine vary greatly Some of the animals listed in the Table may appear to be quite unusual and queer, but that is due to the enterprising activities of my friend Goodall, who one day hired an airplane and went down to Africa (he served as a pilot during the war) and sent home a nice assortment of suprarenals from various animals, including one lion

I do not claim that this list is complete, at all, it just gives some examples picked from the literature and from our own experience, but it is evident from it that the degree of methylation varies practically from 100 per cent down to something in the neighborhood of 10 per cent Why this is so, we do not know It is perhaps superfluous to speculate on the various amounts at this moment, but what I want to draw attention to is the content in man Generally, the methylation in man seems to be around 85 per cent This is not a fixed figure As found by Hokfelt (11) in our laboratory, and also by West (12), the relative amount of nor-epinephrine is much higher in fetal suprarenals In fact, in the early fetal stages, there is practically no epinephrine at all Even at birth, the nor-epinephrine dominates the picture, whereas later on, at greater ages, the relative amount of epinephrine increases In one case, operated upon at the age of 70, the percentage of epinephrine was over 90

Shorr. Is this total epinephrine, Dr von Euler?

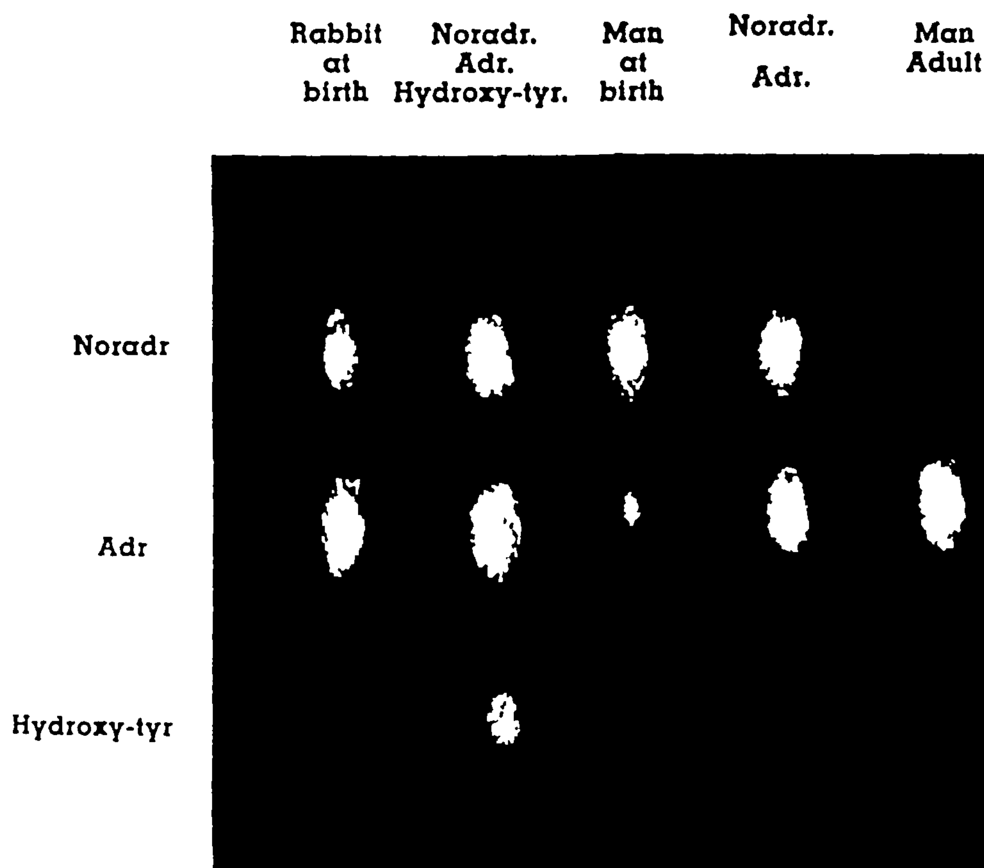


FIGURE 27 Paper chromatogram Extracts from suprarenal glands of adult men, newborn infant, and newborn rabbit compared with pure solutions of hydroxytyramine, epinephrine, and nor-epinephrine Solvent butanol-HCl Time 26 hours Temperature + 26° C Photographed during exposure to ultraviolet rays after developing with potassium ferricyanide Reprinted, by permission, from Hokfelt, B Noradrenalin and adrenalin in mammalian tissues Distribution under normal and pathological conditions with special reference to the endocrine system *Acta physiol scandinav* 25, *Suppl 92 (1951)*

Baez Professor von Euler, do you have any information on animals with the adrenal demedullated, but with the cortex intact?

von Euler No, we have had no experience with that Would you expect that the cortex would be of importance in the formation of these substances?

Baez I was thinking about your findings of a higher content of nor-epinephrine in the parotid and submaxillary glands Something conditioned these tissues for a higher concentration of nor-epinephrine than is present in the skeletal muscle or the liver, for instance I wonder whether the distribution in the several tissues would become more uniform after adrenal demedullation

von Euler We have not determined the amount of epinephrine and nor-epinephrine separately in these glands after adrenalectomy

peritoneum. The total amount of catechol amines in the ganglia of the dogfish was 9 mg. per gm., which corresponds very well with the amount found in the medulla alone. In the cow, for instance, it is about 10 mg per gram

Nickerson. Dr. von Euler, I recall that West has suggested, from a somewhat shorter series than this, that there is a positive correlation between the cortical-medullary ratio and the ratio of epinephrine to nor-epinephrine Do you find that this relationship continues to hold in your series, or does it break down?

von Euler: It does not seem to hold true. In the whale, for instance, the suprarenal looks to me very much like the suprarenal of the baboon, in general shape and in the relation between cortex and medulla.

Burch: What is the relationship in the whale?

von Euler. I should say that from a whale, a suprarenal weighing 500 gm would have a cortex of about 2 cm or 3 cm. thickness and that the rest of it would be medulla, if I remember rightly from the experiments of Rastgeldi (14).

Green: In the whale, did you isolate the amounts in the cortex and medulla, which might be easily separated.

von Euler: Dr. Rastgeldi did not do that. Figure 27 illustrates the difference in the proportion between epinephrine and nor-epinephrine at birth, and in the adult man. This is taken from Hokfelt's paper (11), showing the chromatograph spots for nor-epinephrine and epinephrine In column 3, nor-epinephrine is far in excess of epinephrine, whereas in column 5, the reverse is true.

Engel· Are there data on the distribution in the rat?

von Euler: In the rat, the distribution is about 85 per cent epinephrine and roughly 15 per cent nor-epinephrine. There is also a shift toward much higher figures of nor-epinephrine in the rat, as shown by Hokfelt (11).

I should now like to comment on the formation of these substances It has been suggested repeatedly that the presence of sympathomimetic amines in tissues, or in nerves, depends on the formation of these substances in the suprarenal medulla, and it has been believed that these substances are merely stored in the nerves and liberated from them. This cannot be the case, however, because after complete adrenalectomy there is no significant difference in the content of nor-epinephrine in the organs. This shows, I believe, that the substances are really formed by the adrenergic neurons themselves

from the gland can easily be collected After centrifugation the plasma can be tested directly on test preparations, without any interference by chemical preparation methods

Engel. Have you tested the effect of the adrenal steroids on the biologic assay itself? I am thinking of several observations which suggest that treatment of animals or patients, with cortisone or ACTH, will potentiate certain effects of nor-epinephrine, such as that on the blood pressure.

von Euler. I do not think it will influence the assay, since all the estimations are made against a standard in each preparation; but it is, of course, of great interest for the mechanism of action of these substances

As regards the secretion of these hormones from the suprarenal, we have been able to confirm the findings of Cannon, and his associates, that the secretion at rest is quite small Often it is hard to demonstrate it, or to get a reliable figure for the output from the suprarenal at rest However, even quite small degrees of stimulation of various kinds induce a secretion, as is well known Such influences are some anesthetics, such as chloralose and ether, asphyxia, sensory stimulation of any kind, and so on Inducement of circulatory pressor reflexes is one factor which also increases the output (18,19)

Shorr. Dr von Euler, do you have any evidence that pressor substances, such as renin or pitressin, will increase the output?

von Euler I do not know whether such experiments have yet been done with this method

Shorr Or epinephrine, itself?

von Euler We have not done that I think it would be an interesting point, since one could compare the output of the two substances.

Loewi All this was demonstrated long ago Epinephrine, when injected, does not influence the output of epinephrine from the adrenal medulla To my knowledge, only histamine, potassium, and acetylcholine stimulate the epinephrine secretion from the adrenal medulla Acetylcholine has the same effect also on the heart

Shorr Does the injection of epinephrine stimulate the production of acetylcholine-like substances?

Loewi Nothing is known about this, why should it?

Shorr As a part of the homeostatic mechanism

Nickerson As Dr Loewi just mentioned, I think the only place that is known to occur is in the heart Certainly, epinephrine injections will cause a vagal release of acetylcholine

We suspect that the relatively high epinephrine content in these glands is caused by chromaffin cells

Shorr These are salt-maintained adrenalectomized rats?

von Euler. They were, if I rightly remember, salt-maintained There were also some experiments on cats.

Shorr. How were they maintained?

von Euler They received no treatment They were sacrificed on the third or fourth day.

One question which has bothered us considerably is the significance of the presence of small amounts of epinephrine in the tissues It has been suggested that the adrenergic neurons produce epinephrine in addition to nor-epinephrine It has been shown by Peart (15) that after nerve stimulation, the venous blood from the spleen may contain small amounts of epinephrine as well as nor-epinephrine This, of course, does not mean that the nerve itself produces epinephrine

We have had some experience with the distribution of the two substances after degeneration of the nerves In the salivary glands, for instance, after cutting the nerves and letting them degenerate, the content of nor-epinephrine is very low, but the epinephrine does not fall off in the same proportion (16,17).

It seems, from these experiments, that epinephrine may be formed by other structures rather than by the adrenergic nerves themselves Whether this formation is in chromaffin cells, present in various organs, we cannot tell At least it is one possibility It has never been shown definitely that the adrenergic nerves also produce epinephrine

Alterations in the distribution of the two substances can be found after subjecting an animal to various operative procedures, or to injections of various substances After hypophysectomy, for instance, the relative amount of epinephrine increases in the organ After treatment with ACTH, either in normal or in hypophysectomized animals, there is a decrease in the epinephrine In thyroxin-treated animals, there is a marked increase in the nor-epinephrine content (11) We do not know the precise meaning of these results They require a good deal of further investigation, so I shall not comment upon them any more

As regards the liberation of these substances it has been shown repeatedly, since the classical experiments of Loewi and of Cannon and his associates, that they can be released by stimulation of the adrenergic nerves It is easy to demonstrate the presence of these hormones in the suprarenal vein, in which the total venous output

Loewi. It is claimed by some scientists that epinephrine does not stimulate the anterior lobe directly, but rather some secretory cells in the hypothalamus, and that their secretion passes through the portal system to the anterior lobe and stimulates its secretions The whole question is not yet decided

von Euler. I might briefly mention a few results which were obtained by the Austrian group working with Professor Brucke, in Vienna (21) In the cat they studied the output from the suprarenal gland under the effect of carotid occlusion, and also of direct electrical stimulation of the hypothalamus, and found that the proportion of epinephrine rose considerably if they stimulated the hypothalamus These results are certainly interesting, and might suggest that there are two kinds of fibers going to the gland one group perhaps leading to the secretion of nor-epinephrine and another group to the secretion of epinephrine.

Nickerson Dr von Euler, were these studies on adrenal vein blood, or on peripheral blood, where material from both the adrenal medulla and peripheral sympathetic nerve endings would be picked up?

von Euler The suprarenal venous blood was tested directly Folkow and I (19) have made a few experiments of the same kind, and with similar results Stimulation of the central end of the sciatic nerve also leads to an increased proportion of epinephrine

Burton In such an experiment, would one have to measure the arterial concentration, and work with the arteriovenous difference between epinephrine and nor-epinephrine in order to rule out the factor that the concentration in the whole circulation might be increased?

von Euler In the peripheral blood, the concentration would be of quite a different order, perhaps one-hundredth of that in the suprarenal vein

Moe What concentrations are actually found in the adrenal vein?

von Euler After carotid occlusion, there would be about 0 5 µg of nor-epinephrine per ml. in the cat At rest, perhaps one-fourth of that amount would be found

Knisely Is there any chance that the neuron secretes this concentration, and is the fiber to which we have been referring a little tube which carries it down?

von Euler In the adrenergic nerve fiber?

Knisely Yes

von Euler. Raising the pressure in the carotid sinus will release the homeostatic vasomotor reflex, of course. In that way, it might even inhibit

Burton Have you any data on stress situations emotion, cold, and so on? Have they been shown to increase the secretion of epinephrine?

von Euler Cold has been shown to increase the secretion

Loewi: Every stress does.

von Euler Some experiments by Abe and his co-workers (20) showed, in the exteriorized suprarenal vein, that such kinds of stress as hard work – the dog was made to run very fast for a long time – increased the output If it were a trained dog that ran for a small stretch, there was very little increase Therefore, it depended upon the degree of stress on the animal.

Shorr I am still bothered by Dr Loewi's statement that epinephrine per se would not produce any kind of discharge It should stimulate the adenohypophysis to the discharge of ACTH Then ACTH might alter the output of epinephrine from the gland. Isn't that true?

von Euler I do not know We have done only two experiments so far on that, and we were not able to show any effect of injected ACTH on the output.

Shorr What was the chronology of those experiments, how soon after the injection were they done?

von Euler One and three hours

Haist An increase in epinephrine output, when epinephrine is given, would be contrary to the usual effect of administration of a hormone on the output of the hormone by the gland would it not? In most endocrine glands, if the hormone of the gland is administered, the hormonal output of that gland diminishes

Shorr That is true I just wondered whether this stimulation by ACTH would be a self-perpetuating circumstance

Loewi It was demonstrated many years ago that any stress stimulates the epinephrine secretory center situated in the hypothalamus The stimulus is then transmitted to the adrenal medulla, and its secretion is provoked The epinephrine in its turn stimulates the secretion of the anterior lobe Is that clear?

Shorr. Yes, it is clear I am reminded of what Dr Fremont-Smith says about questions: "Never worry about asking a foolish question, nobody knows what wisdom it will call forth in someone else."

Haist: Where does the hypophysial-portal circulation come into the picture?

spleen, lower than in the heart, probably because it is possible to denervate the spleen more completely.

Cotzias Dr von Euler, we have been encouraged to ask even foolish questions, and I want to ask one You have to extract these agents quite vigorously from the tissue in order to get them out, do you not? In addition, they are rather slow to diffuse. Therefore, I wonder whether it would be possible, in a tissue which is known to contain a great deal of these agents, to find out actually in what intracellular component most of these agents might be concentrated Specifically, I was thinking of the technique of Palade, in which a very high concentration of sucrose is used as the medium for dispersion It is a mild treatment and the hypertonicity is such that very little swelling, or similar phenomena, takes place, there is very little water movement One might possibly, I presume, be able to test the fractions with your procedure and find out if a certain cytologic component is blessed with more, or with less, concentration of these agents than another one Do you think that is a foolish suggestion, in terms of all the washings that take place?

von Euler Not at all You mean, different structures within the cell?

Cotzias Within the cells You see, you can get clean nuclei, completely clean mitochondria, a fraction which consists of a mixture of broken cells, nuclei, mitochondria, all sorts of things that one cannot further fractionate with this method, and a supernatant which consists of soluble substances With enzymes and with various other agents, it is possible to get a clear-cut picture of where in the cell the agents might be located (23)

von Euler I believe this has proved to be useful for demonstrating the position of enzymes, and certain high molecular substances On the other hand, I think that small molecular substances, such as catechols, which are readily diffusible, would be more difficult to place unless they were bound to high molecular substances There is also the fact that they are apparently destroyed, to a certain extent, at the point of release not as fast as acetylcholine, but sufficiently fast to prevent any greater leakage out into the blood stream

Knisely This is particularly interesting in connection with the spleen, because it has a very precise internal controlling apparatus for the distribution of the blood, which could hardly operate without a control system, almost like the finger controls of playing a piano (24,25,26,27)

von Euler We have not found any organ with such a high con-

von Euler: I should think that possibility would be excluded by the very slow propagation of the substance in such neural tubules, if they exist Regarding the prolonged effects of stimulation of adrenergic nerve with a suitable frequency, one would not think it possible that enough material could be shoved down to the nerve endings

Knisely That would be my first assumption, but I am still curious Has anyone cut off the end, say, of the splenic nerve, and stimulated the upper part to see what comes out? This may be a foolish suggestion, but perhaps not

von Euler: Åstrom and I (22) have done a few experiments on isolated splenic nerve trunks, and on stimulation at the end There is, in some cases – I would not say in all – a release of nor-epinephrine at the other end What the significance is, I could not say

Knisely Is the substance produced at the far ends of the nerves, rather than at the neuron body?

von Euler The substance is actually produced by the whole neuron It is found in the whole neuron, but to a much higher extent in the nerve endings, possibly due to the presence of necessary substrates, or coenzymes, or something like that, which accelerate the process of formation.

Shorr. That was clear from your work on the spleen

Loewi Why do you think so?

von Euler The figures obtained from the splenic nerve trunk, and the spleen itself, indicate that the concentration of nor-epinephrine in the nerve endings must be much higher than in the trunk itself

Nickerson That is excluding the possibility that some of the material in organs, such as the spleen, is stored outside of nervous tissue?

von Euler: That is one possibility, but we have no evidence for that. There is always a quite good correlation between the number of adrenergic fibers going to the organ and the nor-epinephrine content of the organ

Nickerson: The sympathomimetics may be formed by nerves, but not stored in them. If all of the nor-epinephrine in the spleen were in the sympathetic nerve endings, it would probably itself form an isotonic, or hypertonic, solution

Stead: If the spleen is denervated, is there the same drop in tissue concentration as when the heart is denervated?

von Euler: Yes The content goes down to practically zero in the

is just a fine strand of fibers leading to the kidney, much less in proportion to the weight of the organ than for the spleen.

Burton. Have you ever looked at the thymus? This is a wild hope, but in view of its connection with the pituitary-adrenal axis, it would be interesting

von Euler: Yes. but I must say it was a great disappointment. The amounts of epinephrine, and nor-epinephrine, are very small in that organ On the other hand, the content is quite high in lymph glands.

Nickerson: One might postulate a relationship between the fine control of blood distribution in the spleen, and its rich content of nor-epinephrine. However, my impression is that aside from preventing massive contractions, denervation does not really cause much breakdown in splenic function I wonder if Dr. Knisely can tell us how much derangement of the splenic circulation occurs following denervation.

Knisely· I do not know at all

Shorr· With respect to what?

Nickerson With respect to the opening and the closing of shunts and the distribution of blood. Grossly, the denervated spleen appears to function normally, except that it does not pour out a mass of red cells under sympathetic stimulation. The reason I raise this question is that I think it is dangerous to conclude that because of the high nor-epinephrine content of the spleen, nor-epinephrine is responsible for the precise control of the splenic circulation.

Burch Have you studied any abnormal spleens or diseased spleens, such as in a patient with a lymphoblastoma?

von Euler. We happened to get a spleen from a young boy with some blood disease I cannot say in detail what the symptoms were, but there was occasion, apparently, for removal of the spleen The spleen came to us fresh from the operating table, and we extracted it in the usual way We found absolutely no nor-epinephrine in it at all

Cotzias Was it very large spleen?

von Euler I did not have that impression

It would be interesting, in regard to the production of catechol amines from the suprarenal glands, to investigate their liberation in shock That has not been done, I believe, at least a differential determination of the epinephrine, and the nor-epinephrine component has not been made I think, in all experiments of this type, it is necessary to differentiate between the two since they are not released in a parallel way

tent of nor-epinephrine as the spleen. It is an outstanding organ from that point of view.

Fine Where does the bone marrow come into the picture?

von Euler. The bone marrow does not contain any measurable quantities. I have not been able to get really reliable figures

Shorr: That brings up one point, Dr. von Euler. We have always been thinking about the adrenal as a source of release, and have examined adrenal vein blood Thinking in terms of some of the problems we have encountered, I wonder whether similar studies have been done on the spleen under a variety of circumstances, such as after ACTH, pressor agents, or what not?

von Euler. Dr. Shorr, it is relatively easy to demonstrate the Cannon-Rosenblueth effect by stimulating the splenic nerves Nor-epinephrine leaks out into the blood stream in sufficiently large amounts to be demonstrated in the same animal by a rise in blood pressure.

Knisely. What are the effects of these substances on liver and liver circulation? The spleen is just upstream from the liver, so the liver must get the highest concentration from the splenic blood. Are there special effects on the liver?

von Euler. I think Professor Hermann Rein would like to answer that, but I cannot venture into that rather intricate field.

Fine. Is there any evidence that nor-epinephrine is inactivated in the liver?

von Euler: Both epinephrine and nor-epinephrine are inactivated by the liver. When epinephrine is infused through the liver, it is destroyed relatively rapidly

Fine I should think, purely on empirical grounds, that it is a necessary provision of nature, since presumably the very high blood concentration of nor-epinephrine is for the purpose of mobilizing blood To flood the circulation with nor-epinephrine on such occasions might even be detrimental, unless the liver were able to take care of the excess.

von Euler: The liver is certainly effective in that respect.

Baez Is there any other organ which has adrenergic fibers in amounts comparable to the spleen? If so, what is your explanation for finding a much larger concentration of nor-epinephrine in the spleen?

von Euler: I do not know of any organ with the same great supply of adrenergic nerve fibers as the spleen. For instance, if one compares the nerves leading to the liver, the nerve bundle is quite small for that large organ. The same is true for the kidney. There

effective than nor-epinephrine, but when larger doses were tried, that ratio was much less. The simultaneous administration of choline, acetylcholine, or methionine increased the effectiveness of nor-epinephrine, but not that of epinephrine. It was considered possible that when large amounts of nor-epinephrine are in the circulation, some of it may be transformed to epinephrine, and that the administration of methyl donors facilitates this process (31).

von Euler There is a possibility of methylation, which is hard to exclude However, this mechanism does not seem to be present to any great degree Otherwise, one would expect to find large amounts of epinephrine in the tissues

Another interesting thing about pheochromocytoma is that in cases where the tumor contains almost exclusively nor-epinephrine, the tumor is situated outside the suprarenal or loosely connected to it, whereas in those cases in which it contains epinephrine and nor-epinephrine, it is emerging from the medulla Therefore, the two types seem to be originating from two different kinds of cells. This has, incidentally, given a hint as to the position of these tumors, since the output in urine reflects very well the proportion of nor-epinephrine and epinephrine in the tumor In a case where there is exclusively nor-epinephrine in the urine, the surgeon can be advised to look for the tumor outside the suprarenals, and usually somewhere along the aorta

Goldenberg· I am sorry to have to disappoint you, Dr von Euler, but we have found that rules in pheochromocytoma hold only as long as the series is small, with a larger series, they become invalid In our last eleven cases, in all of whom the urinary excretion of catechol amines was studied, only one tumor was extra-adrenal and ten were adrenal Of these adrenal tumors, three showed predominantly nor-epinephrine, and one contained practically no epinephrine at all For weeks, chromatograms were run, just to show traces of epinephrine in this tumor In this patient's urine we found, in addition to nor-epinephrine, a hydroxytryamine-like substance in large amounts, but no trace of epinephrine This was an intra-adrenal tumor (32)

I think that as far as the idea of the separate secretion of nor-epinephrine and epinephrine goes, we may have to wait for more proof I admit that Brucke's investigations (33) are quite suggestive, but the differentiation of small shifts in the epinephrine/nor-epinephrine ratio by bioassay still seems problematic It is noteworthy that Gaddum (34) abandoned the use of the primary bioassay (modified de Jalon method) for the differential estimation

Dr Burton suggested that I should also say something about the production of catechol amines in adrenal medullary tumors. the so-called pheochromocytoma It was shown by Pamela Holton (28), in England, that some pheochromocytoma contained rather high proportions of nor-epinephrine. In two cases she found approximately 90 per cent, in a third about 50 per cent, and the rest was epinephrine. We have been able to confirm this (29), as has Dr Goldenberg (30) and others It seems to be quite often the case that the tumor contains practically nothing but nor-epinephrine In other cases, there is a higher content of epinephrine, in addition, but there is always more nor-epinephrine than in the normal gland

There would perhaps be a possibility of finding out whether there are different kinds of cells producing nor-epinephrine and epinephrine if one really looked carefully into these tumors. Our histologists and pathologists have not so far been able to tell us whether there is any difference in, for instance, a rabbit's supra-renal, producing only epinephrine, and in a pheochromocytoma that is producing only nor-epinephrine

Moe· I presume the tumor cells do not have any innervation from so-called preganglionic fibers

von Euler. I think not

Moe· It would be very strange if they did You also remarked that in the fetus the content of nor-epinephrine is much higher than of epinephrine, is that right?

von Euler That is right

Moe Can it be that choline is the methylating agent, and that the humoral agent which causes the liberation of epinephrine from the normal medulla is the agent which adds on the methyl group? Henry Hoberman had a fanciful theory about this ten years ago when I was in Otto Krayer's laboratory in Boston. We set up an assay procedure with the hope of distinguishing between epineph-rine and nor-epinephrine, using the relaxation of the constricted bronchi as an evidence of epinephrine action, and the blood pres-sure response of the same animal as a measure of the sum of nor-epinephrine and epinephrine. I won't go into detail, because we did not have much in the way of results However, we were trying to demonstrate the possibility of methylation of nor-epinephrine by the addition of choline or acetylcholine.

Nickerson· I recall that someone was studying the effect of epinephrine and nor-epinephrine on the mouse pupil It was found that if minimal doses were used, the epinephrine was much more

replacement which has to be given in order to maintain a normal blood pressure postoperatively.

I remember one case we studied in which we had to give 0 3 mg of epinephrine per minute intravenously for forty-eight hours, in order to keep the systolic blood pressure at about 110 mm Hg.

Loewi. What kind of case was it?

Shorr. A pheochromocytoma after operation

Moe· A special mechanism for the release need not be postulated, it seems to me, because if these tumor cells are synthesizing epinephrine and nor-epinephrine continuously, it has to get out. It cannot be contained within the cell forever It could leak out in the direction of the concentration gradient

Loewi: Not necessarily, it could also be destroyed within the cell.

Moe Surely

Shorr· It is an accepted fact that the tumors are independent of the usual type of stimulation for this release

Nickerson I get the impression from the pharmacologic methods of inducing attacks in patients with pheochromocytoma that there is some correlation between vasodilation in the tumor tissue, which allows the pressor material to be washed out, and the production of an attack. It may be largely a mechanical process

Zweifach· What is the physiologic significance of differences in the content of nor-epinephrine and epinephrine of various tissues?

von Euler. In my opinion the nor-epinephrine is at least the predominant, and may be the exclusive, mediator of the adrenergic nerves, whereas epinephrine probably serves other purposes

Zweifach You presented in several of your illustrations the comparative content of nor-epinephrine and epinephrine for different tissues, some tissues having rather large amounts, relatively speaking Is there any significance attached to these figures, aside from the type of innervation, with reference to function or physiology?

von Euler I should think that the amount of nor-epinephrine signifies the degree of adrenergic nervous innervation In some organs, such as a striated muscle, or the lung, the supply of adrenergic nerves, is apparently rather small, whereas in other organs an extensive adrenergic nerve supply is required In such a case, there will be a large amount of the substance What governs the adrenergic nerve supply to the organ is another question

Shorr. Dr von Euler, have you ever studied the effects of adrenergic blocking agents on the production of epinephrine by the adrenal medulla?

of epinephrine and nor-epinephrine in mixtures of these compounds, and replaced it by chromatographic separation of the compounds, followed by bioassay of the single eluted compound (35).

It is hard to believe that there are different cells containing epinephrine or nor-epinephrine, because an individual starts in fetal life with a gland producing nor-epinephrine only, and comes to have, by slow transition, a gland producing a mixture which is predominantly epinephrine. I questioned pathologists about the differentiation of tumor cells producing, predominantly, epinephrine or nor-epinephrine. No luck.

von Euler: They have not found the answer.

Goldenberg: One must look either for different cells, or for different transmitters in the adrenal medulla, if an independent secretion of epinephrine and nor-epinephrine is assumed.

von Euler: Dr. Goldenberg, do these tumors really emerge in the medulla, or are they just adjacent to the medulla?

Goldenberg: I had always thought that the extra-adrenal tumors were more frequent, since we had observed a higher percentage in our previous series. However, in the last eleven cases we were not lucky; we had just one extra-adrenal. It had a high percentage of epinephrine.

von Euler: All I can say is that in our thirteen cases, it has been true that when there is nor-epinephrine in the urine, the tumor has been extra-adrenal.

Loewi: Do these tumors produce and release nor-epinephrine?

von Euler: They release, in many cases continually, one or both of the substances.

Loewi: This is obviously not a release from nerves.

von Euler: No.

Loewi: Is anything known about substances which provoke the release?

Goldenberg: Histamine does, for example, it has been used as a test. We tried to block the release by antihistamines. It always worked for two days but not on the third day.

Burch: It may be released by massage.

Goldenberg: Mechanical factors succeed so rarely that it seems possible that a humoral factor may release it. Unfortunately, we do not know how the attacks are precipitated.

Shorr: There are cases in which the tumor is readily accessible to palpation, and in which a small amount of palpation invariably produces a rise in blood pressure. However, the extent of the continuous release may be indicated by the amount of epinephrine

replacement which has to be given in order to maintain a normal blood pressure postoperatively.

I remember one case we studied in which we had to give 0.3 mg of epinephrine per minute intravenously for forty-eight hours, in order to keep the systolic blood pressure at about 110 mm Hg.

Loewi. What kind of case was it?

Shorr. A pheochromocytoma after operation

Moe A special mechanism for the release need not be postulated, it seems to me, because if these tumor cells are synthesizing epinephrine and nor-epinephrine continuously, it has to get out. It cannot be contained within the cell forever It could leak out in the direction of the concentration gradient

Loewi Not necessarily, it could also be destroyed within the cell

Moe. Surely

Shorr It is an accepted fact that the tumors are independent of the usual type of stimulation for this release.

Nickerson I get the impression from the pharmacologic methods of inducing attacks in patients with pheochromocytoma that there is some correlation between vasodilation in the tumor tissue, which allows the pressor material to be washed out, and the production of an attack It may be largely a mechanical process

Zweifach What is the physiologic significance of differences in the content of nor-epinephrine and epinephrine of various tissues?

von Euler In my opinion the nor-epinephrine is at least the predominant, and may be the exclusive, mediator of the adrenergic nerves, whereas epinephrine probably serves other purposes

Zweifach You presented in several of your illustrations the comparative content of nor-epinephrine and epinephrine for different tissues, some tissues having rather large amounts, relatively speaking Is there any significance attached to these figures, aside from the type of innervation, with reference to function or physiology?

von Euler I should think that the amount of nor-epinephrine signifies the degree of adrenergic nervous innervation In some organs, such as a striated muscle, or the lung, the supply of adrenergic nerves, is apparently rather small, whereas in other organs an extensive adrenergic nerve supply is required In such a case, there will be a large amount of the substance What governs the adrenergic nerve supply to the organ is another question

Shorr Dr von Euler, have you ever studied the effects of adrenergic blocking agents on the production of epinephrine by the adrenal medulla?

von Euler: No, we have not done any such experiments.

Loewi. Quite recently some experiments of this kind were performed I have here a preprint of a preliminary note. Shall I read it?

Shorr: Yes, do

Loewi The following is from "Sympatholytic Drugs and the Adrenal Glands," by Mary F Lockett (36,37)

> "Bulbring (1949) first demonstrated the methylation of noradrenaline to adrenaline by minced suprarenal gland, and found that added adenosine triphosphate was essential for this methylation
>
> "Lockett (1952) confirmed Bulbring's work, and extended it by showing that the minced suprarenal glands demethylate adrenaline to noradrenaline in the absence of added ATP
>
> "Ergotoxine ethanesulphonate, tolazoline, (priscoline), hydrochloride, or dibenamine, added to suspensions of adrenal glands, suppress the demethylation of adrenaline and cause methylation of nor-adrenalin in the absence of added ATP Sympatholytic drugs therefore act like ATP in this respect, and make ATP no longer essential for the methylation of noradrenaline *in vitro*
>
> "Similar action results from the *in vivo* use of sympatholytics Suspensions made from the adrenal glands removed from animals after 'adrenaline reversal' had been induced with any one of these three sympatholytics, methylated noradrenaline to adrenaline in the absence of added ATP, suspensions of control glands, removed from the same animals prior to the injection of a sympatholytic drug, demethylated adrenaline to noradrenaline in the absence of added ATP, and methylated noradrenaline to adrenaline in the presence of either added ATP, or an added sympatholytic drug"

Shorr It does not answer the question of total production, but of a shift in equilibrium, which might conceivably be the mechanism, rather than different cells If there were an equilibrium reaction between epinephrine and nor-epinephrine in the medulla, which could be influenced in one direction or the other, then it would not be necessary to postulate different cells for doing different things

Haist: I should like to ask if it is possible to alter the proportion of epinephrine and nor-epinephrine in tissues by diet For example, if a choline-deficient diet were administered, would it have any effect on the proportion of the two?

von Euler: We have had no experience with that, I think it is a question which is worth looking into

Burch. Dr von Euler, have you studied the influence of continued prolonged stimulation, such as might occur in stress situations, to see if there is any exhaustion phenomenon, and if so, whether epinephrine or nor-epinephrine becomes exhausted first?

von Euler. We have just started some experiments but the number of them is so small that I would not like to comment upon them I would say that so far we have not been able to detect any consistent alteration in the content of the organ The stimulation periods, however, have been up to only twenty minutes.

Moe. Is this nerve stimulation?

von Euler It is nerve stimulation of the spleen

Moe. There were some old experiments, I think from Edmund's laboratory, which involved giving physostigmine, and atropine, and then several large doses of acetylcholine. There was repeated liberation of the medullary hormone, and I believe they demonstrated a significant reduction in the content There was, of course, no differentiation between epinephrine and nor-epinephrine in those experiments

von Euler On splanchnic stimulation, it is possible to empty the gland to a certain extent Insulin is still more effective, as shown by Hokfelt in the rat (11)

Moe. However, I think that without the addition of physostigmine it is impossible

Loewi Elliott, long ago, strongly diminished the content of the adrenal medulla by splanchnic stimulation without using physostigmine (38)

Moe This was prior to the demonstration of nor-epinephrine in the adrenal

Fine Are the amounts of epinephrine and nor-epinephrine decreased at the same time?

von Euler On splanchnic stimulation, yes

Fine Does that suggest that epinephrine is the precursor of nor-epinephrine in the tissues?

von Euler I would not say so

Nickerson Before we leave the note which Dr Loewi read, I should just like to add a word of caution This is an interesting observation and may ultimately prove to be important However, at the present time, it probably should not be taken as an indication of the mechanism of action of these drugs

Loewi I fully agree with you

Nickerson In the case of Dibenamine, we know that the compound is a potent inhibitor of certain sulfhydryl enzymes The epinephrine-nor-epinephrine interconversion may well be dependent upon the action of such enzymes The inhibition of some enzyme involved in demethylation could cause the apparent shift in the direction of the reaction We shall have to know more about this

von Euler. No, we have not done any such experiments.

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Shorr Yes, do.

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TABLE X

Exercise minutes	O_2-consumption liters per min	Maximum lactic acid mg %	Total catechol amine output* µg per min of work	Per cent epinephrine of total catechols	Excess output µg/min
Rest			0 03	17	—
Rest			0 05	38	—
20	1 85		0 03	28	0
20 5	2 1		0 03	21	0
19	2 4		0 04	16	0
20	2 4		0 07	42	0 02
17	3 1		0 06	57	0 01
21	3 8		0 18	16	0 13
16	4 0	47	0 43	18	0 38
21 . .	4 1	47	0 50	22	0 45

*The total catechol amine output per minute is calculated by using a resting output value of 0 05 µg per minute

reaction before we can say that this effect of Dibenamine is related in any way to the other actions of this drug

EXCRETION OF CATECHOL AMINES IN URINE

von Euler: I should like to give a short survey of some observations on the excretion of the sympathomimetic amines in urine, and a brief outline of their actions. I know that Dr Ahlquist is going to present some more precise data as to the mechanism of action.

With regard to the excretion in urine, it was shown by Holtz and co-workers (10), that normally a certain amount of sympathomimetic amines is present in the urine He inferred from his experiments that these amines were epinephrine, nor-epinephrine, and hydroxytyramine, which has been confirmed by us (39).

The normal excretion in man appears to be, according to our estimation, about 4 to 8 μg of epinephrine per day, on an average, and about 20 to 40 μg of nor-epinephrine per twenty-four hours. If one follows the excretion in a hospitalized patient, for instance, who is living under rather regular conditions, the output in urine is quite constant On the other hand, if the subjects are up and doing their daily work, there might be alterations which would exceed those figures.

Loewi. Were the determinations made directly or, according to Richter, after hydrolysis of the urine?

von Euler These figures were obtained after hydrolysis of the urine. We used twenty-minute hydrolysis with sulfuric acid at pH 1 5, at 100 degrees centigrade

Loewi. Was Richter's idea correct?

von Euler It was correct, except that Richter did not, at that time, demonstrate the presence, at normal times, of such substances, or their spontaneous output That was first shown by Holtz

Green. What technique did you use to demonstrate the presence of these substances?

von Euler· We used essentially the same technique that we used for demonstrating their presence in organs After hydrolysis of the urine, aluminum sulfate, and then sodium hydroxide, were added to form the precipitate which absorbs the catechol amines The precipitate was then dissolved in acid and the aluminum salts removed It has been shown that during certain conditions the output may be greatly altered One of the first series of experiments we made was on the effect of muscular work

von Euler: That is a very good point This particular subject did not show an increased output until his work went up to about 3 or 4 liters of oxygen per minute However, in untrained subjects a similar increase could be noticed even at 2 to 3 liters per minute Therefore, it appears to be the degree of stress on the subject which determines the degree of output of catechol amines

Shorr. Have you similar observations following ACTH?

von Euler We have only one case, I have not mentioned it because I felt it might be premature to do so

Green Are there data on the time course of this output, with respect to a period of work?

von Euler. Unfortunately, no The necessary minimum amount of urine can hardly be produced in less time than, say, fifteen minutes, and that makes a detailed study somewhat difficult, especially since one cannot be sure about the complete emptying of the bladder before and after It would make the figures rather uncertain

Stead Do you have figures on the excretion during motionless standing, or a similar type of stress?

von Euler No, we have not

Shorr Dr Selkurt, would you tell us something about what happens to renal function and flow, and to excretion, during these periods?

Selkurt. It is a well-known fact that renal function tends to go down during exercise, and also that the actual urine output goes down There may be a combination of reduced renal blood flow and alteration in normal output of ADH (antidiuretic hormone) in terms of the final effect on volume of urine

Shorr Therefore, Dr von Euler was really justified in waiting for a considerable time after the period of actual work?

Selkurt Yes, I should say so Of course, it leads to an interesting question in terms of the mechanism of excretion. If the catechol amine is simply filtered, then a reduction of urine volume would not matter because it would be concentrated more highly in the final specimen

Shorr But this is still referred to in terms of output per minute, so the concentration would not matter

Selkurt Then it could be influenced only by alteration in filtration rate

Shorr In other words, the changes may be very much greater, because Dr von Euler collected urine at a time when the filtration rate was low, so that he would not be getting as much from the

Table X gives an example of a series of determinations in a well-trained subject. The work done was controlled by measuring the oxygen consumption There is no definite alteration in the catechol amine output as long as the muscular work does not exceed 2 4 liters of oxygen consumption per minute. On the other hand, if the amount of work per unit of time is increased considerably, up to about 4 liters per minute, which of course is quite heavy work, then the output figures go up steeply (40) As shown in Table X, at 4 liters per minute, the output was about 0 5 μg. per minute· approximately a tenfold increase

Nickerson: What was the duration of each of these collection periods and work periods?

von Euler· The work periods lasted from five to twenty minutes, and the collection of urine was usually once an hour In those cases the calculation was made on the basis that the normal output in rest should be 0.05 μg per minute

Nickerson. They were not consecutive periods?

von Euler. No, they were on different days The next to last column shows that the percentage of epinephrine did not change very much Therefore, both nor-epinephrine and epinephrine would be secreted in increased amounts

This, perhaps, should be commented upon a little further When the adrenals have been removed, the epinephrine output in urine falls practically to zero (40) That means that the source of the epinephrine output is chiefly the suprarenals On the other hand, in adrenalectomized patients, the nor-epinephrine output does not change to any great degree, which shows that the nor-epinephrine is not derived from the suprarenals to any great extent but comes from other sources, presumably the adrenergic nerves

This is of some interest because it shows that during heavy muscular work there is a considerable increase in the activity of the adrenergic nerves At the same time, formation and release of epinephrine from the suprarenals takes place These are probably quite different procedures

Remington Did you say adrenalectomized patients?

von Euler Yes These patients were maintained on a daily dose of 50 mg of cortisone We followed the catechol amine output regularly

Engel· Would there be any difference in the output with muscle exercise between an individual in poor condition and, say, an athlete?

von Euler. That is a very good point This particular subject did not show an increased output until his work went up to about 3 or 4 liters of oxygen per minute However, in untrained subjects a similar increase could be noticed even at 2 to 3 liters per minute Therefore, it appears to be the degree of stress on the subject which determines the degree of output of catechol amines

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Selkurt Then it could be influenced only by alteration in filtration rate

Shorr In other words, the changes may be very much greater, because Dr von Euler collected urine at a time when the filtration rate was low, so that he would not be getting as much from the

blood. Also, during the post-exercise period, inactivating mechanisms may be operating to reduce the amount appearing in the urine. Therefore, these figures might be regarded as minimal.

Stead Do you know to what level these materials have to be raised in the blood stream in order to obtain this amount of urinary excretion?

von Euler: Yes; Dr Luft and I (41) have studied the effect of intravenous infusion of nor-epinephrine on urinary excretion, and found that between 1 5 and 3.3 per cent of the amount given was excreted in the urine per unit time These figures, I believe, are in approximate agreement with those obtained by Dr. Goldenberg, who found 2 per cent Is that right?

Goldenberg: May I tell you what we found later? As we became more courageous, and increased the amounts of infused nor-epinephrine, we found that the excretion increased to 4 per cent There seems to be an increase of excretion ratio with increasing load. Some cases of pheochromocytoma, with paroxysmal hypertension, who have had a single severe attack lasting for fifteen minutes only during a 24-hour period, excrete amounts comparable with that of a patient who has had persistent hypertension due to pheochromocytoma for 24 hours. That means that if the circulation is suddenly flooded with very large amounts, an even higher percentage of excretion than 4 per cent occurs I do not think that this percentage change would really matter in the case of work, because the total amounts are still small and would not influence the results

Burch: Was the 1 5 per cent of the total amount collected over a given period of time?

von Euler. It was 1 5 to 3 3 per cent

Burch Did you measure the blood level?

von Euler No, we have no method of measuring the blood level unless it is very high Dr Lund, in Copenhagen, specialized in the fluorometric method and found that during normal conditions the level, less than 1 μg per liter, is too low to be estimated in peripheral blood. But in cases of pheochromocytoma he succeeded in demonstrating nor-epinephrine several times, and arrived at figures which are probably nearly correct

Moe What are those figures, approximately?

von Euler. In the one case which we observed jointly, he found on an average, 36 μg per liter of plasma (42).

Moe: What would be the expected excretion rate in such an individual? Perhaps you have determined this in the same manner?

von Euler Yes, we determined the excretion rate It was about 100 µg per hour against the usual figure of 1 to 2 micrograms.

Moe Would you say 2 µg. per minute?

von Euler Something like that.

Shorr We have some observations on bloods from a few pheochromocytoma patients which may be of interest We first established, in a rat meso-appendix preparation, the threshold response to topical application of epinephrine, and then instead of epinephrine we applied the blood topically Samples drawn from patients in a quiescent state were equivalent to epinephrine dilutions of one part in three or four million Bloods drawn during induced attacks assayed at one in one million or less, indicating roughly a three or fourfold increase in whatever was giving the epinephrine-like response, and suggesting that the meso-appendix can be used to measure these high levels, at least approximately

Nickerson: During the past year, Dr Peacock from Bristol, England, who was working at the University of Michigan, carried out some epinephrine and nor-epinephrine assays in our laboratory, using the chromatographic method described by Crawford and Outschoorn (35), and both fluorometric and animal assay of the eluates Although the figures, in general, tended to be somewhat higher than expected, i.e , normal values of 1 to 2 µg per 100 ml. of blood, the material which was obtained seemed, by all criteria, to be sympathomimetic, and the amounts obtained correlated very well with the responses of patients with Raynaud's disease to cold At least in this application the procedure seemed to give a fairly good measurement of what was going on in the body

Burch Do calculations of the clearance suggest that there was reabsorption of that which filtered through the glomeruli?

Nickerson No, this work was done on peripheral blood, and on renal vein blood obtained by catheterization He obtained considerably higher concentrations in the latter

Burch Did calculations indicate reabsorption of large quantities?

Moe I think there was about 50 per cent reabsorption from this Copenhagen-Stockholm patient, without correction for possible binding to plasma protein

von Euler Of course there are the recent estimates by Weil-Malherbe and Bone, reported at the Chemical Congress in Paris, in which they estimated, by means of the fluorometric method, after condensation with ethylenediamine, the amount of catechol amines to be normally about 0 3 µg per 100 ml in the peripheral blood (43)

Nickerson: Was this determination preceded by chromatography?

von Euler: No.

Nickerson: This was the direct coupling reaction?

von Euler. Yes. They absorbed it first with an aluminum hydroxide to purify it

There have also been some other observations on the output of sympathomimetic amines in the urine. In a series of experiments, Dr. R. Luft and I (44) showed that after insulin, 0 1 International Unit per Kg intravenously, the output of epinephrine was increased ten times In this case, the nor-epinephrine was not increased at all. In fact, there was a small but statistically significant decrease in nor-epinephrine This shows that there is a distinct difference between the processes which determine the output of epinephrine and of nor-epinephrine, as might be expected.

Moe. Couldn't this just mean that the insulin provokes a discharge of the adrenal medulla, but not of the sympathetic system in general?

von Euler Yes

Loewi: It is a hypothalamic stimulation, so it must act on the nerves

Moe I agree

von Euler That's right In insulin-resistant cases, such as acromegalic patients, there is no increase in the epinephrine output

Nickerson. Weil-Malherbe has published an abstract of experiments in which he found that the administration of insulin in humans caused quite a sharp reduction in the amount of circulating epinephrine (45) I am not sure how it fits into the picture

von Euler. I believe, Dr Nickerson, that what they estimated was not epinephrine That is the only inference I think one could draw Dr Weil-Malherbe himself is quite careful not to state that it is epinephrine He says it is an epinephrine-like substance which might include not only epinephrine and nor-epinephrine but also some other things

Nickerson I am not sure that the reduction in active material in the peripheral blood, and the increase in excretion of hydrolyzable material, are necessarily incompatible with their being basically the same substance It may be that production is increased but that utilization, or conjugation, is increased even more What you are measuring in the urine reflects the total production, and what is measured in the peripheral blood is the difference between production and utilization, or inactivation

von Euler. That seems to me hard to understand, because if hypoglycemia leads to an increased output of epinephrine in the suprarenal gland, I am sure it would show in the blood.

Loewi. Is the output in urine ten times the normal?

von Euler Yes These observations are in agreement with changes in the circulation observed by Luft and myself, and by Ernstene and co-workers (46) They showed that the increase in heart rate, and the alterations in the diastolic and systolic pressure, would be in agreement with epinephrine excretion

In the acute stage of coronary thrombosis there is regularly an increased secretion of nor-epinephrine, about two or three times normal That has been found by Forssman and co-workers (47). Luft and I found that in postural hypotension, which is perhaps the first example which has any direct relation to the shock problem, the output of nor-epinephrine and epinephrine is very greatly reduced In two cases, carefully observed and clinically diagnosed and verified as true cases of postural hypotension, the output of nor-epinephrine was reduced to about one-fifth to one-tenth of normal, and the epinephrine was reduced in about the same way These patients did not respond to insulin with an increased epinephrine secretion, or, if they did, only to a very small degree Apparently, in this particular condition, the ability of the adrenergic nervous system to produce nor-epinephrine is impaired to such an extent that the vasomotors do not secrete, or do not liberate enough of the substance

Moe Were these patients unduly sensitive to insulin?

von Euler Yes, these two were

Moe I suppose this was spontaneous postural hypotension and not the result of surgical intervention?

von Euler They were both spontaneous cases

Burton May I suggest that one has to be a little more definite about postural hypotension, because there are several clinical examples of it Perhaps one should call it classical orthostatic hypotension, which is, I think, the accepted name of the type you are speaking of There are other types in which it is due to the pooling of blood, or to cholangiomas It would not apply there, would it?

von Euler Dr Luft, with whom I have been collaborating on this, was very careful in selecting those two cases

Stead Did they have signs of other autonomic nervous dysfunctions, such as sweating and fixation of the heart rate?

von Euler. Dr. Luft told me that they did not sweat normally

Burton: Years ago, I studied a case of classical orthostatic hypotension, who had normal sweat gland responses. It is not necessary, in this type of hypotension, that the sweating mechanism be involved

Goldenberg. May I know the absolute values of nor-epinephrine secretion in these two cases?

von Euler. They were less than 5 μg per 24 hours of nor-epinephrine

Goldenberg. We found such low values in hypertensives following thoracolumbar sympathectomy

von Euler If one is allowed to calculate the concentration of nor-epinephrine in blood from the infusion rate, which admittedly involves certain assumptions as to the inactivation rate, one might arrive at a figure of $10^{8 5}$ on infusion of 14 μg. nor-epinephrine per minute. If one also assumes that the relation of the blood level and the output is of the same order, which may not be completely true, then one would arrive at a figure on the order of $10^{9 4}$ in normal peripheral blood, that is to say, a concentration which would probably not have any definite action Whereas in pheochromocytoma, the concentration would be something about $10^{7 7}$, which would give a very marked circulatory result This figure of $10^{7 7}$ is speculative but it fits in quite well with figures found by Lund Therefore, we have some degree of confidence in the order of magnitude of these figures

Figure 28 shows the result of analyses of the urine in 500 cases, which were chiefly essential hypertension The output in urine in about 70 per cent of the cases was normal In the rest, about 30 per cent, there was a doubtful increase in the first column, and then a definite increase in the last five columns, representing quite a small fraction of the total number of these cases

What inferences can be drawn from these figures I cannot say at present They would tend to show that the level of circulating nor-epinephrine in the majority of cases is not increased above the normal

Engel What percentage of normal cases gets up in the range of the hypertensive patients?

von Euler We have not seen any normal case secreting over 86 4 μg per 24 hours These figures may appear a little bit queer, but they are calculated on the output per minute, i e , 0 06 μg per minute

In the case of pheochromocytoma referred to previously (42), the urine contained such high amounts that injection of 0 1 ml of the

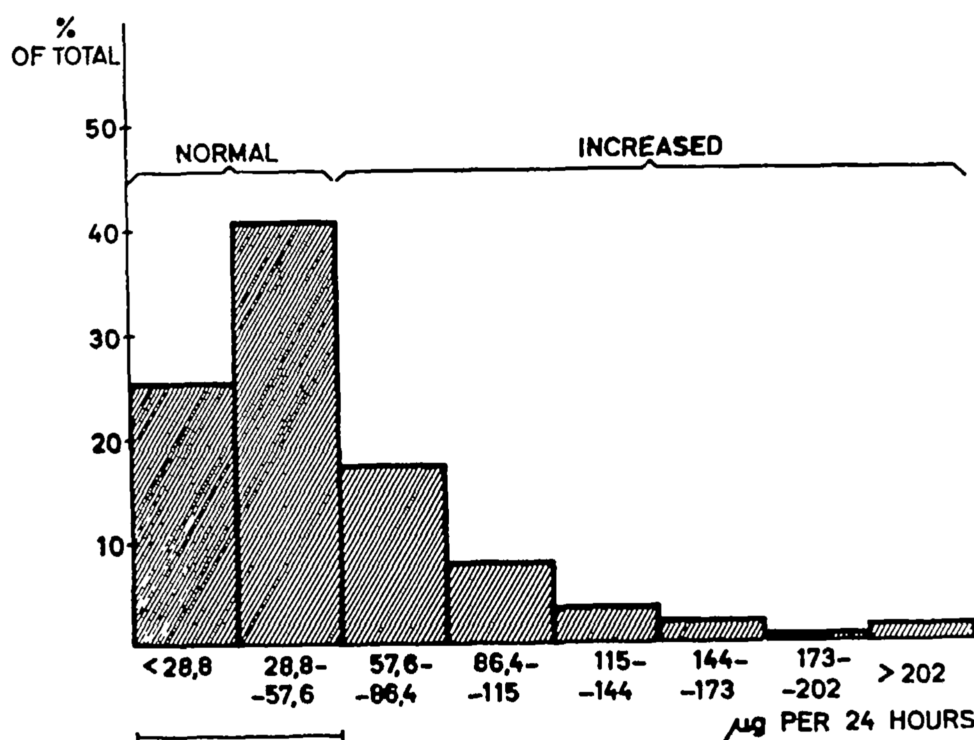


FIGURE 28 Excretion of nor-epinephrine in urine in 500 cases of essential hypertension

untreated urine produced an effect on the cat's blood pressure which was clearly visible, and equal to the effect of 0 3 μg. of nor-epinephrine (Figure 29) The total amount in this case was 2400 micrograms It can also be seen that the effect of epinephrine is of quite a different type

As a screening method, simple injections of untreated urine may be used On the other hand, if the excretion is considerably less than 0 5 μg per ml, it might be difficult to assay it in that way, without further purification

Loewi May I come back to a question raised before? You found that in muscular work there was quite a strong excretion of both nor-epinephrine and epinephrine, but that in the case of hypoglycemic stress, only epinephrine was excreted How do you explain this difference?

von Euler We have tried to explain it this way during muscular work, there will be a vasodilation in the working muscles, which might be greater than the corresponding increase in flow, and therefore a compensatory vasoconstriction must appear in other parts of the body in order to maintain the blood pressure This can be seen, for example, after exclusion of the carotid sinus and the other blood

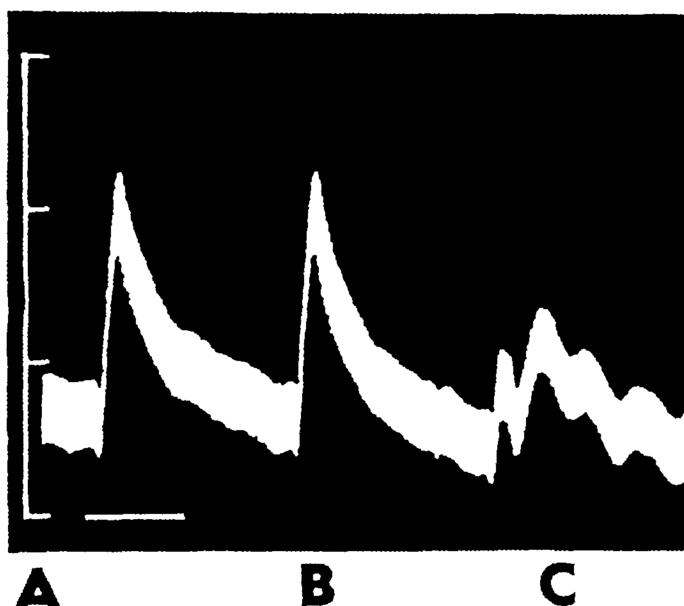


FIGURE 29 Cat, Nembutal, Blood pressure I V injections of untreated urine of case of phaeochromocytoma (G P) B P 130-190 mm Hg Time 1 min

A 0 1 ml. urine G P
B 0 3 μg *l*-nor-epinephrine
C 0 4 μg *l*-epinephrine

Reprinted, by permission, from von Euler, Lund, A , Olsson, A , and Sandblom, P Noradrenalin and adrenalin in blood and urine in a case of pheochromocytoma. *Scandinav J Clin & Lab Invest* (In press)

pressure-controlling mechanisms If one induces moderate muscular work in a normal anesthetized animal, there is no change in blood pressure On the other hand, if one excludes these regulatory mechanisms, the same degree of work produces a profound fall in blood pressure, thus showing that normally the blood pressure homeostatic mechanisms are operating during muscular work (48).

It has also been shown that after sympathectomy in man, muscular work may lead to a fall in blood pressure Thus we believe that in these cases there is a strong increase in the activity of the vasomotor nerves in certain regions, leading to increased formation and excretion of nor-epinephrine, which is thus concerned with the vasometer part of the reaction

Green· Do you, by any chance, have any observations on the output of epinephrine preceding or during migraine headaches?

von Euler· No, we have not.

RESPONSES IN VARIOUS ORGANS TO INJECTIONS OF EPINEPHRINE AND OF NOR-EPINEPHRINE

The pioneer paper of Goldenberg and his associates (49) showed that nor-epinephrine, administered by infusion in man, raises the

systolic and diastolic blood pressure, and thus the mean pressure, whereas it has been shown that epinephrine raises the systolic pressure but may, in moderate doses, even lower the diastolic pressure, thus leaving the mean blood pressure only slightly increased

Ahlquist Dr. von Euler, I should like to put in the record the doses, 0 11 to 0 4 μg per Kg. per minute, which Dr Goldenberg used, because these small doses, by continuous intravenous infusion, are different from pharmacologic or physiologic doses and do not necessarily produce the same type of response For example, there was a case in Georgia, done before I went there, in which they were measuring the arterial pressure in a human with the Hamilton manometer The intern inadvertently gave ten times the prescribed dose of epinephrine intravenously, and the pressure rose to a very high level There was typical vagal slowing, and it was difficult to distinguish it from the typical epinephrine response in the more common experimental animals Therefore, I think it is important that the dose be noted

Goldenberg In the case you cite, do you regard the dose as pharmacologic or physiologic?

Ahlquist. As a physiologist, I usually give more of a drug than a pharmacologist does, so I would regard that as a physiologic dose. It is common in the physiologic literature to see doses of epinephrine in the range of 0 5 mg per Kg , and up In the pharmacologic literature, with the exception of some of the classical work on Dibenamine done by Dr Nickerson, the dose seldom approaches that figure I think Dr Nickerson, after his work on Dibenamine, did give large, physiologic doses of epinephrine

Haist That should be called a physiologist's dose rather than a physiologic one

Nickerson I believe the major issue is not whether there is a significant difference between the action of epinephrine and nor-epinephrine Everyone will agree to that The hidden question is whether there is an important difference between the responses of man and those of our experimental animals This has often been implied, but I believe that the major difference is due to a difference in dosage We have studied in fifty or sixty patients the response to 1 0 μg per Kg of synthetic or commercial *l*-epinephrine, given as a single, rapid, intravenous injection The pressor responses are almost identical with those seen with the same dose in cats or other experimental animals The systolic pressure rises 40 to 60 mm , and

there is always a rise in diastolic pressure which averages about 30 mm , and a period of vagal slowing of the heart.

Ahlquist: What I had in mind was that we should not leave this particular point until it has been clearly established that, up to now, there has been found no significant difference in the response to epinephrine and related compounds among experimental animals, whether they are dogs, cats, or humans. The differences appear to be quantitative rather than qualitative

Goldenberg There is a definite difference between man and certain animal species, such as the dog Whatever the range of dosage selected in dogs, consistent hemodynamic differences cannot be shown between epinephrine and nor-epinephrine so far as cardiac output and total peripheral resistance are concerned The fact that there is a striking difference in man in a certain range, and it is a pretty wide range, should not surprise a pharmacologist, dosage and responses must be quantitated and over-all estimates of drug actions cannot be given. Is the same response to digitalis over the whole range of doses expected? One digitalis dose will increase the strength of the heart, and a higher dose will cause ventricular fibrillation

The dose range we studied was pretty wide We infused at the rate of 0 1 to 0 4 μg per Kg per minute This is a dose which is well beyond the physiologic range, although not the physiologist's range, because with nor-epinephrine there is hypertension of, let us say, 200/130 in a normal subject, and with epinephrine a rise of systolic pressure to about 180 mm of mercury

Concerning the actions of epinephrine and nor-epinephrine in man under physiologic conditions, we can definitely state that there is a striking difference, nor-epinephrine increases total peripheral resistance and epinephrine increases cardiac output Going far beyond this amount of nor-epinephrine, as in pheochromocytoma, metabolic actions are encountered which are confined to epinephrine in the physiologic range On the other hand, the excessive amounts of epinephrine secreted by pheochromocytomas may cause over-all vasoconstriction. However, we are not primarily concerned with pheochromocytoma, but with actions in the physiologic range.

Ahlquist: I want to challenge the statement of Dr. Goldenberg, that in the dog you cannot tell the difference between the two Later, I hope to present some data which will show that there is a marked difference It cannot be seen, I admit, on the blood pressure as recorded by the mercury manometer, nor very well on the heart rate However, in certain vascular beds and smooth muscle,

there is a great difference between the two I am sure Dr Green can bear me out on that

Goldenberg I am happy about it What I was referring to were studies by Wegria on cardiac output and the total peripheral resistance in dogs. He was unable to find any difference

von Euler What seems to be of primary interest here is the change in the mean blood pressure by moderate doses I should say doses within the pathophysiologic range The cardiac output, as Dr Goldenberg already has said, is increased by some 25 or 30 per cent with epinephrine in such a dose range, whereas the output is not much altered by similar doses of nor-epinephrine This, of course, means, as Dr Goldenberg has pointed out, that the net action of nor-epinephrine in the body is one of vasoconstriction, whereas the net result of such a dose of epinephrine is vasodilation I think this difference should be stressed, because it appears to be quite a fundamental one I know it is quite difficult to try to introduce that kind of concept to several colleagues. to say that epinephrine produces a net vasodilation It produces opposition in them because it is well known, on the authority of every textbook, that epinephrine is a vasoconstrictor Of course, these figures, just by plain physics, show that it must be a vasodilation

Knisely Dr von Euler, there is the work of Bauer, W., Dale, H H, Poulsson, L T and Richards, D W (50) which shows very clearly that in the dog epinephrine opens the outlet valves of the liver It dilates that set of vessels Since then, there has been a small abstract from our own laboratory showing that certain small sphincters, located where the sinusoids of the liver join the central veins, are caused to open by certain concentrations of epinephrine (51) Thus, the concept of special actions of vasomotor substances in special anatomical places is now ready for wide investigation

von Euler The slowing effect of nor-epinephrine on the heart rate is largely reflex in origin and may not be of any primary interest at the present

The effect of nor-epinephrine on the coronary vessels is indeed of importance Figure 30 is taken from a paper by Smith and his co-workers (52), showing the dilatory effect of nor-epinephrine on the excised coronary artery This dilation even exceeds that of a similar dose of epinephrine At any rate, it has been shown by other techniques also that both these substances cause a dilation of the coronary vessels In view of the picture shown earlier this morning, which demonstrated that the coronary vessels do contain nor-

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heart was measured by a rotameter? In these experiments, nor-epinephrine was found to cause a significant increase in coronary blood flow

Ahlquist. I have reservations regarding all methods of measuring blood flow in the coronary system when using epinephrine or nor-epinephrine These substances change the metabolic and muscular activity of the myocardium. The changes, in themselves, can make great differences in blood flow Dr Shipley (53), and others, who have done a great number of rotameter experiments on coronary flow with epinephrine, will not make a positive statement one way or the other as to whether epinephrine is, or is not, a direct coronary dilator What I mean by direct action is an action not mediated through changes in activity and metabolism of the myocardium

Green· May I make one point in regard to dosage? One microgram of epinephrine intra-arterially in the leg is a potent constrictor, but 1 µg intra-arterially is almost ineffectual in the coronary artery vascular bed The dose has to be increased to almost ten times that amount to have an effect On the coronary circulation, epinephrine tends to produce, first, a greater reduction in flow during systole, and, second, dilation or increase in flow in diastole, which fits in with the concept that the epinephrine vasodilation may be secondary to myocardial stimulation rather than a direct vasodilator effect (54).

Engel What is the effect of epinephrine or nor-epinephrine on the myocardium?

Ahlquist· I do not know, but it makes the heart beat much harder

Engel Has this been studied by *in vitro* techniques? I am interested because of an observation of Burdette's (55,56) In his studies of the oxygen consumption of cardiac muscle in rats, subjected to hemorrhagic or tourniquet shock, there was a striking increase in the oxygen consumption of cardiac muscle slices soon after bleeding, or within the first 30 minutes after removal of tourniquets

When I first saw these results, the question came to my mind as to whether this could be either an epinephrine or nor-epinephrine effect Dr Burdette was kind enough to send me some of his unpublished data in which he was trying to answer this by doing the same experiment in the adrenalectomized rat, and in the adrenalectomized rat given a dose of epinephrine (I do not know whether it was commercial adrenalin or epinephrine) In his results there was a suggestion that the increase in oxygen consumption immediately after injury did not occur in the adrenalectomized animal, but did in the animals given a dose of epinephrine ten minutes

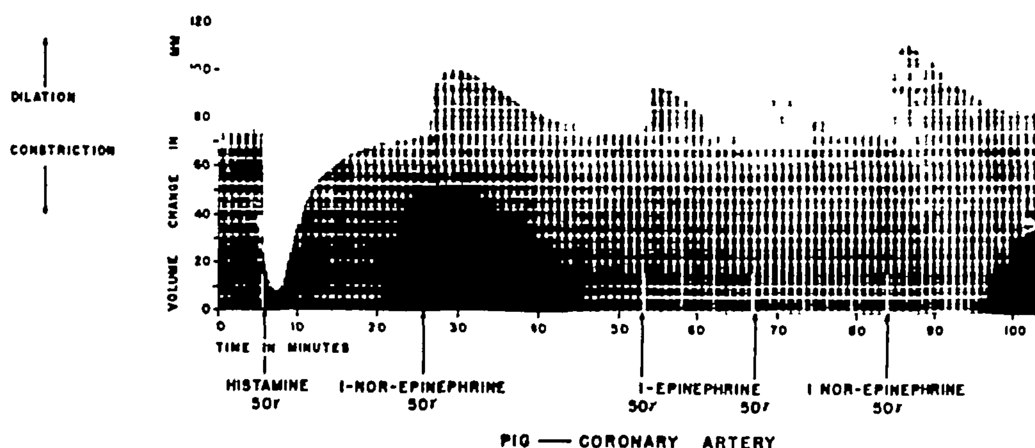


FIGURE 30 Histamine first produced vasoconstriction Vasodilation was then caused by *l*-nor-epinephrine, *l*-epinephrine, *l*-epinephrine again and finally by *l*-nor-epinephrine Note the reproducibility of responses Reprinted, by permission, from Smith, D J, Syverton, J. T, and Coxe, J W In vitro studies of the coronary arteries of man and swine as demonstrated by a new technique, angioplethysmokymography *Circulation* 4, *890 (1951)*

epinephrine, I think it is clear that this is a physiologic substance for dilating the coronary vessels

Ahlquist. Dr von Euler, do you want us to believe that what happens to a large artery is the same as what happens farther out in the vascular tree? This must be a fairly large artery which you are describing, because to use the plethysmographic method it is necessary to have something which you can cannulate and put fluid into It is not an arteriole, and it is not placed where I think the flow is actually controlled Therefore, my personal opinion is that while this is a very good demonstration of what a coronary artery does, it is not a good illustration of what may actually be going on farther out where coronary flow is determined

von Euler I agree, at least in part, with what Dr Ahlquist has said I had some reservations about saying that on the perfused heart, also, there is a vasodilator effect which can be demonstrated However, I quite admit the validity of the objection

Moe Also in reference to Figure 30, do you know, Dr Nickerson, whether the pig has a rise of pressure in response to histamine?

Nickerson I do not know about the pig

Moe These are tremendous doses for the whole pig, I should think Probably it is not a very large pig Are these 50 gamma of epinephrine in a small volume of fluid, or 50 gamma per liter?

Goldenberg May I ask Dr Ahlquist whether he will accept Wegria's experiments* in which the coronary flow of the dog's

*Wegria, R, *et al* Unpublished data

marked constriction that it is almost the equivalent of a coronary artery occlusion (54).

Loewi: Dr. Engel earlier mentioned experiments which I performed ten years ago. I wonder whether it is permissible to refer to papers more than forty years old? In 1905, O B. Meyer kept rings cut from various arteries in oxygenated Ringer's solution and added epinephrine The rings of all the arteries were constricted, except those from the coronary arteries, which in most cases were dilated (60) Later, these results were often confirmed I don't know whether experiments of this kind are still valid

Moe That was Dr Ahlquist's objection to the big coronary artery. that it was a large vessel and not an arteriole

Ahlquist I do not think the large arteries control the flow. I am sure there are a lot of things that go on in the aorta. for example, the size of the aorta changes, but I am sure it does not affect mean pressure very much. It may change the pulse contour, which we do not want to get into here because the proponents of that are still arguing about what it means

von Euler. May I add just one thing? Precisely in these large coronary arteries, nor-epinephrine was shown to be present in the extracts, which probably means that they are innervated by vasomotor nerves producing nor-epinephrine Perhaps it will be admitted that nor-epinephrine is the predominant factor in the action of the vasomotor nerves, and that probably in the heart its actions would then be fairly satisfactorily limited.

Turning to some other types of actions of these substances, it may be appropriate to look at the metabolic actions first

It has been well known for a very long time that epinephrine acts on the metabolic process in various ways, as seen by its effect on the blood sugar, on the blood lactic acid, on the oxygen consumption, and so on The question has been whether nor-epinephrine has more or less the same action. It seems from the data available in the literature that nor-epinephrine does have a similar action, but it has also been shown that it is necessary to give a somewhat higher dose in order to produce the same effects.

Table XI illustrates this point (61). Here are listed, somewhat arbitrarily, a few actions on metabolic processes and the substance responsible for primarily vascular actions It should not be forgotten, of course, that nor-epinephrine also influences the activity of the smooth muscle in general, in such places as the intestinal tract and the uterus. On the other hand, epinephrine seems to act predominantly on metabolic processes which are accelerated to a higher

before he sacrificed them. I was wondering whether there was any precedent for believing that there might be such a metabolic effect of epinephrine, or nor-epinephrine, on the myocardium.

Ahlquist I think there are many references in the literature to the fact that oxygen consumption of the heart is very definitely increased by very small doses

von Euler. Yes, Gremmels and others have shown that.

Engel. Is this oxygen consumption of the myocardium measured *in vitro*? There is the problem of blood flow if the oxygen consumption is measured *in vivo,* which might conceivably modify the oxygen consumption.

Ahlquist: I presume it would occur *in vitro* just as well as *in vivo,* because with the isolated perfused heart, which is supplied with nothing but Ringer's solution and some glucose, there will be the same response of the heart. It is not necessary to have blood there, or any oxygen, except what is dissolved in the Ringer's solution

Green: There were several papers which showed that the efficiency of the heart goes down in terms of oxygen consumption per unit of work done under the influence of epinephrine (57)

Moe. That, by the way, depends very strongly on the conditions under which the heart is allowed to respond to the epinephrine If the heart is allowed to diminish in size in response to epinephrine, and I presume also to nor-epinephrine, the oxygen consumption is not increased so much, the work also is not increased so much. If an attempt is made to keep the fiber length constant, in the Starling fashion, and an equivalent dose of epinephrine is given, the work goes up enormously, and the oxygen consumption may not increase in proportion as much as the work Therefore, under those conditions an increase in the efficiency of the myocardium can result

von Euler I think it is Gremmels who has done some experiments on this, and has demonstrated in the heart a so-called wasteful oxygen consumption after epinephrine (58)

Moe Gollwitzer-Meier has done similar experiments, with the same results But in both cases, the volume of the heart, that is the fiber length, was not controlled The heart was allowed to diminish in size (59)

Burch. I should like to ask a question for my own edification Is there any reliable coronary vasoconstricting agent?

Ahlquist Pitressin

Burch I had forgotten about this action of pitressin

Green. Pitressin injected into a coronary artery will produce such

degree than with similar amounts of nor-epinephrine There is thus an indication of functional differentiation between the two systems. the adrenergic sympathetic system acting through nor-epinephrine, and the suprarenal medulla acting primarily by secretion of epinephrine.

I am stressing this because there is frequently found in the physiologic and clinical literature the expression. "the sympatho-adrenal system," which I think is rather misleading, since it seems to imply that these two systems work parallel and produce the same kinds of changes in the body. I do not believe this is true They can vary, as we know, independently of each other, and I think one serves the circulatory functions primarily, and the other serves metabolic functions

Shorr: Dr. Bradley has information regarding the striking differences in the effects of epinephrine and nor-epinephrine on the excretion of sodium and potassium

von Euler· Just one further word before Dr Ahlquist gives a more extensive review of the mechanism of action, and that is the effect on the nervous system. It has been shown by Marrazzi, and by others, that epinephrine inhibits the transmission in peripheral synapses This effect is not produced by similar doses of nor-epinephrine, which is about five times less active in this respect That would seem to indicate that here also a metabolic process of some kind is involved

I should like, too, to draw attention to the well-known fact that the subjective effects of epinephrine and nor-epinephrine are quite different in man After an injection of, say, 1 mg of epinephrine, most subjects have a peculiar feeling of anxiety and apprehension. After nor-epinephrine, these very characteristic subjective feelings are not in evidence There may be a vague impression that something is going on in the circulation, but no feelings of the kind produced by epinephrine are noticeable This may indicate, too, that epinephrine acts on some metabolic processes in the cortex, which are not influenced by similar doses of nor-epinephrine

Green Could you clarify what you meant by peripheral synapses?

von Euler Peripheral autonomic synapses, such as in the superior cervical ganglion

Moe This is probably a ridiculous question, but I have always been curious about the difference between epinephrine and nor-epinephrine in regard to the feeling of apprehension Is it true that, in severe emotional stress such as fright or extreme anger, the adrenal medulla itself discharges? In other words, in severe emo-

TABLE XI

Subject	Test	Route and Dose	Activity Ratio Nor-epinephrine/ Epinephrine	Authors
Rabbit	Oxygen consumption	i v inf 10 μg /Kg min	1 11	Lundholm (1950)
Man	Splanchnic oxygen consumption ml /sq m/min	i v inf 0 10 μg /min	1 5	Bearn, Billing, Sherlock (1951)
Man	Hepatic glucose output mg /sq m/min	i v inf 0 10 μg /min	1 6	Bearn, Billing, Sherlock (1951)
Man	Capillary glucose mg /100 ml	i v inf 0 10 μg /min	1 5	Bearn, Billing, Sherlock (1951)
Rat	Blood glucose		1 5	Ronzoni, Reichlin, (1950)
Man	Peripheral venous lactic acid	i v inf 0 10 μg /min	1 6	Bearn, Billing, Sherlock (1951)
Rat	Adrenal ascorbic acid	s c	1 5	Nasmyth (1949)
Man	Eosinophil count in blood		1 6	Humphreys, Raab (1950)

apprehension when the patient is under the influence of Dibenamine?

Nickerson The effect is not as great, although the tachycardia may be more pronounced. The Dibenamine certainly does not prevent the apprehension, but it seems to reduce it.

COMPARISON, IN VARIOUS ORGANS, OF THE RESPONSES TO INJECTIONS OF EPINEPHRINE AND NOR-EPINEPHRINE

Ahlquist Up to now, Dr von Euler has led us along a very attractive and tempting path to the end of the nerve He has proposed that from the adrenergic nerves comes nor-epinephrine and that from the adrenal medulla comes primarily epinephrine, subject to species variation as he has shown I should like to make an approach from the other end, starting from the effector cell and working back from the neuro-effector junction to see if we come out with the same answer

First, a definition of terms is appropriate, so that we all understand that we are talking about the same thing Dr von Euler has faithfully tried to use the terms epinephrine and nor-epinephrine, rather than adrenalin and nor-adrenalin I shall speak of epinephrine and "arterenol," arterenol being the name for nor-epinephrine which I prefer When I speak of arterenol, unless it is qualified definitely, I am speaking of synthetic levo-arterenol bitartrate made by Winthrop-Stearns Epinephrine is the synthetic levorotatory bitartrate

As a very brief introduction, I should like to bring you up to date on what we have done in the past (63) In Figure 31 are shown five sympathomimetic amines and their chemical relationships arterenol (nor-epinephrine), a compound I call methylarterenol, which is also known as Cobefrine, epinephrine, a compound which I call simply methylepinephrine, and finally, N-isopropylarterenol, known also as isopropylnorepinephrine, Isuprel, or Aludrine These were used in their racemic forms because three of them are not yet available in their levorotatory forms They were first compared in arterial pressure on dogs, cats, and rabbits They were also compared on isolated vascular beds, as shown in Figure 32

This Figure shows the peripheral resistance plotted against time There is first a comparison in the renal artery At the arrow, the amines were given intra-arterially, vasoconstriction is indicated by the rise in peripheral resistance The *l*-epinephrine, which was used as a standard throughout, gave the greatest increase in peripheral

tional stress, will there be an outpouring of epinephrine itself, whereas in response to ordinary circulatory stresses, such as a change in posture, will the carotid reflexes, governing the adrenergic constrictors which liberate nor-epinephrine be evoked? Any individual mature enough to serve as a subject for the administration of large doses of nor-epinephrine, would certainly, at one time or another, or at many times, have been exposed to severe emotional or frightening situations And I wonder if the cardiovascular picture produced by epinephrine might not evoke, as a sort of conditioned reflex, the feeling of apprehension

von Euler The experiments by Dr Cannon, and his co-workers, have clearly shown that during fright, or other kinds of severe emotional stress, there is an increased output of epinephrine Everybody who has had the experience of an intramuscular, or even intravenous, injection of epinephrine, remembers that very peculiar and disagreeable feeling The subject probably states that he feels very much the same as he would feel if he had been driving a car and had just narrowly escaped an accident

Remington Do you think that it is any more than the feeling of palpitation?

von Euler. Definitely It is something which can hardly be described As Dr Moe points out, even the strong vascular reflexes, attended by a great number of impulses in vasomotor nerves, would not produce that kind of subjective feeling

Burton This subjective feeling is not present during heavy exercise, is it? That is rather curious

Remington. Perhaps not during the exercise However, it would appear immediately afterward, while the tachycardia still persisted, and there was nothing to distract the attention

Goldenberg. Hemodynamic studies were done on patients who were mildly frightened I think they were done in your department, Dr. Stead, by Dr. Hickam (62) These patients showed the same circulatory changes as though they had been given epinephrine: an increase in pulse rate and a drop in total peripheral resistance.

Nickerson: I have a feeling that the cardiac response, although it is probably not the only factor, is important in producing the subjective reaction We have observed this subjective response in hypertensive patients who were given Dibenzyline and developed a marked orthostatic hypotension with tachycardia If the tachycardia was prevented with a ganglionic blocking agent, the feeling of apprehension was much reduced

Moe. Does a large dose of epinephrine produce a feeling of

apprehension when the patient is under the influence of Dibenamine?

Nickerson The effect is not as great, although the tachycardia may be more pronounced The Dibenamine certainly does not prevent the apprehension, but it seems to reduce it

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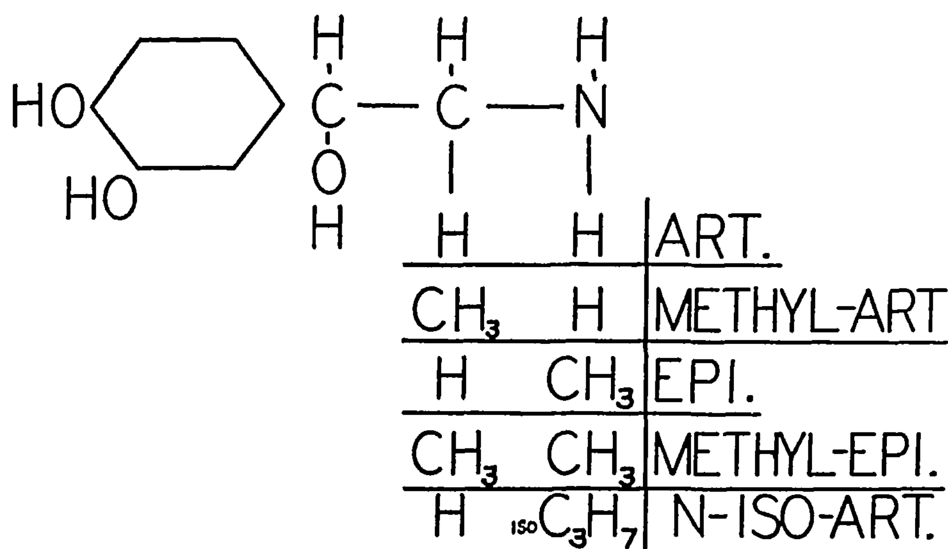


FIGURE 31 The chemical relationship of the sympathomimetic amines most closely related to epinephrine

resistance The racemic epinephrine was second, arterenol was third, and methylarterenol was fourth. Methylepinephrine and N-isopropylarterenol had little effect on the peripheral resistance in the kidney

In the mesenteric artery, ignoring the *l*-epinephrine, epinephrine and arterenol were about equal In the femoral artery, a much greater difference was seen With epinephrine, the primary vasoconstriction was followed by a secondary vasodilation, but with arterenol there was no secondary dilation The methylepinephrine and N-isopropylarterenol, produced typical vasodilation in the femoral artery.

Table XII summarizes the earlier work Here the amines are arranged in the order of their comparative activity The most active are to the left, the least active to the right Vasoconstriction, as judged from renal resistance in the dog and arterial pressure in the rabbit, uterine excitation, in rabbit uterus mainly, the effects on the nictitating membrane and *dilator pupillae* in the cat, ureteral constriction, which I know some will object to, determined in the intact rabbit; and intestinal inhibition in dogs, cats, and rabbits, all follow the same order of activity The effects listed at the bottom of the Table vasodilation, judged by the arterial pressure response after an adrenergic blocking agent, and by the secondary effect in the femoral bed; uterine inhibition in the rat, guinea pig, and virgin cat, and myocardial excitation, determined on the perfused rabbit heart, and on the pulse contour of the dog, follow a different order of activity

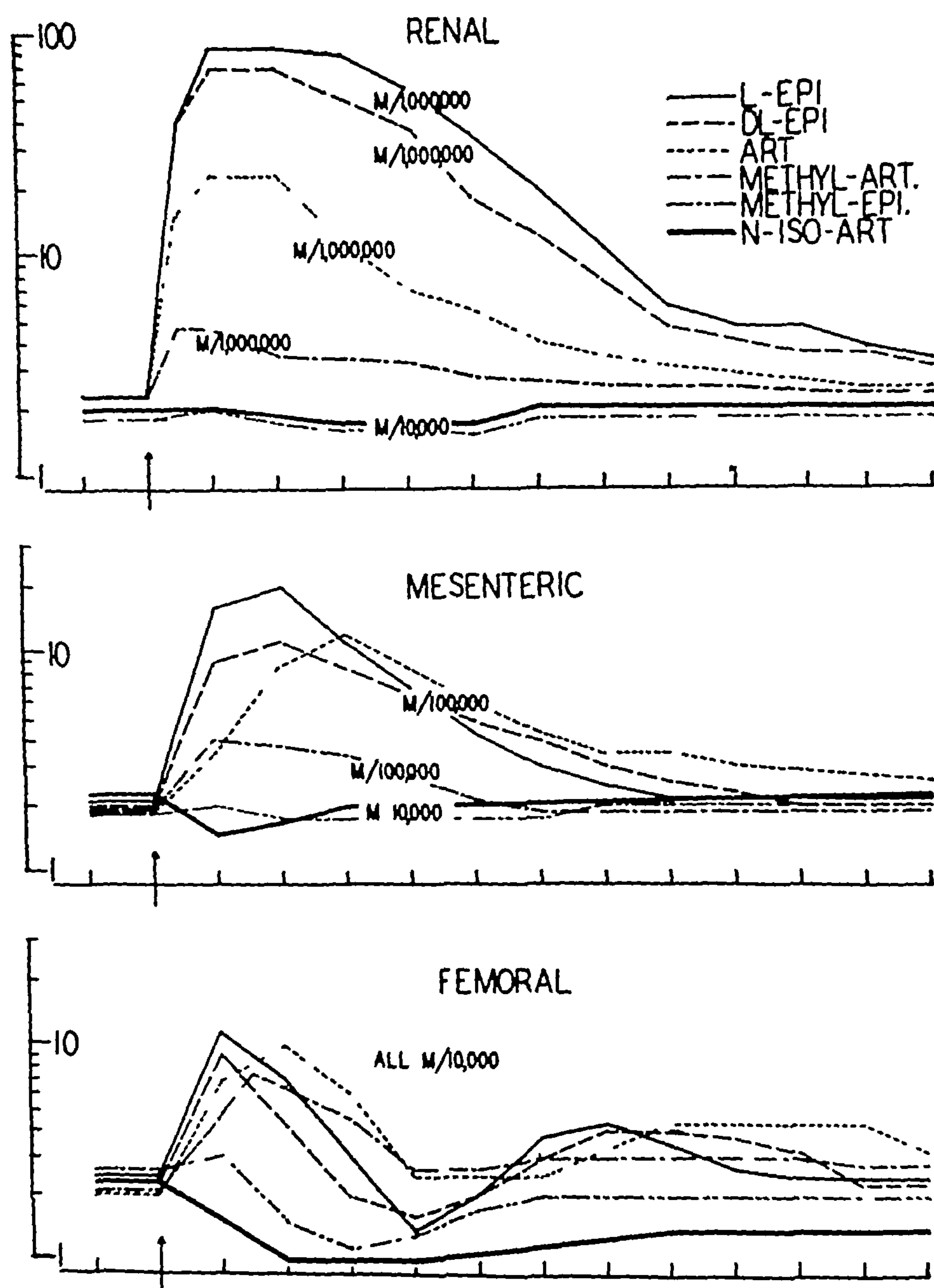


FIGURE 32 Comparative actions of the amines on vasomotor resistance (VR) in the renal, mesenteric and femoral vascular beds of dogs Ordinates VR plotted logarithmically for convenience only (VR = Pressure in mm Hg-20/blood flow in ml /min), abscissae, time marks at 10 second intervals The amines were injected intra-arterially as 0 1 ml of the concentration shown Reprinted, by permission, from, Ahlquist, R P , A study of the adrenotropic receptors *Am J Physiol.* **153**, *586 (1948)*

TABLE XII

Summary of the Relative Order of Activity of the Amines

Receptor	Order of Activity: Most active →					Least active
Vasoconstrictor . . .	l-epi.	dl-epi.	art.	methyl-art.	methyl-epi.	N-iso-art
Uterine excitatory .	l-epi.	dl-epi.	art.	methyl-art	methyl-epi.	N-iso-art.
Nictitating membrane excitatory	l-epi.	dl-epi.	art	methyl-art.	methyl-epi.	N-iso-art.
Dilator pupillae excitatory	l-epi.	dl-epi	art.	methyl-art.	methyl-epi	N-iso-art.
Ureteral excitatory .	l-epi	dl-epi.	art.	methyl-art.	methyl-epi.	N-iso-art
Intestinal inhibitory	l-epi	dl-epi.	art	methyl-art	methyl-epi.	N-iso-art.
Vasodilator	N-iso-art.	l-epi.	methyl-epi	dl-epi.	methyl-art.	art.
Uterine inhibitory .	N-iso-art.	l-epi.	methyl-epi.	dl-epi	methyl-art	art.
Myocardial excitatory	N-iso-art.	l-epi	methyl-epi.	dl-epi.	methyl-art.	art

Reprinted, by permission, from Ahlquist, R P A study of the adrenotropic receptors. *Am J Physiol.* **153,** *586 (1948)*

When I found these two orders of activity, I proposed the existence of two adrenotropic receptors, through which the sympathomimetic substance acts on the effectors Some sort of receptive mechanism must be postulated because the muscles are anatomically similar, the nerves are anatomically similar, but a different response is obtained from nerves going to one muscle and nerves going to another muscle This receptive mechanism is an old concept, introduced by Dale (64) about fifty years ago. For want of better terms, I called these the *alpha* and *beta* receptors, the *alpha* all being excitatory with the exception of the gut, the *beta* all being inhibitory with the exception of the heart It is questionable where the intestinal inhibition should go It doesn't go with vasodilation, or uterine inhibition. And it is also questionable where myocardial excitation belongs It obviously doesn't belong with vasoconstriction and that group It may be that there are more than two kinds of receptors

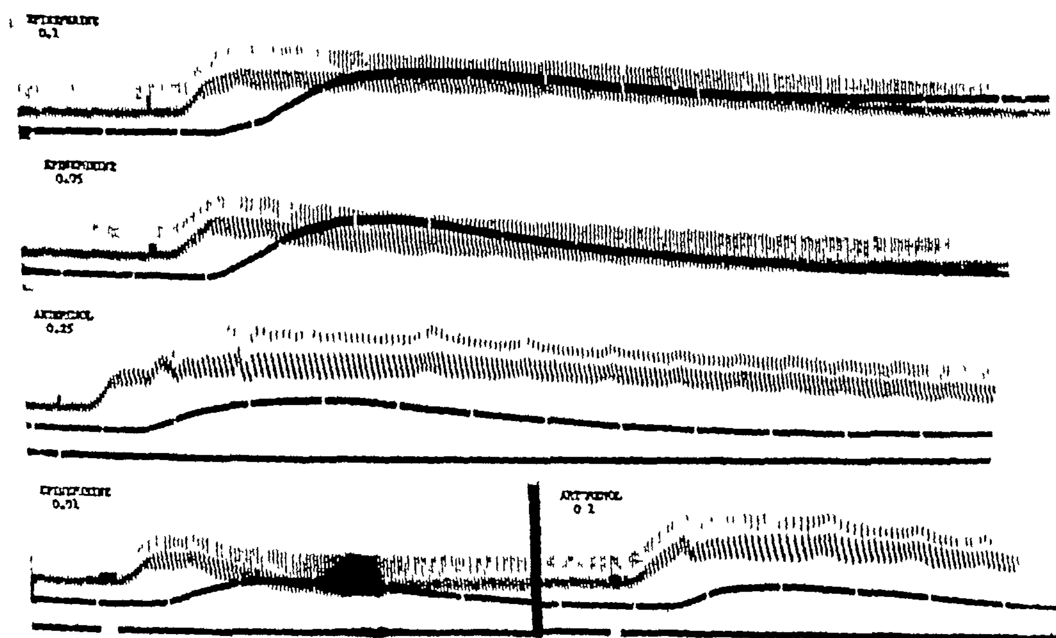


FIGURE 33 Arterial pressure and splenic contraction in the dog Time breaks at 10 second intervals Dose, 1 = 0 01 ml of M/1000 solution/Kgm administered intravenously

The next four Figures illustrate the main thesis I wish to discuss Figure 33 is a record of the arterial pressure and splenic contraction * The contraction of the spleen is recorded by a "difficult" method

*The studies on *levo*-arterenol were supported by a grant from the American Heart Association, Inc

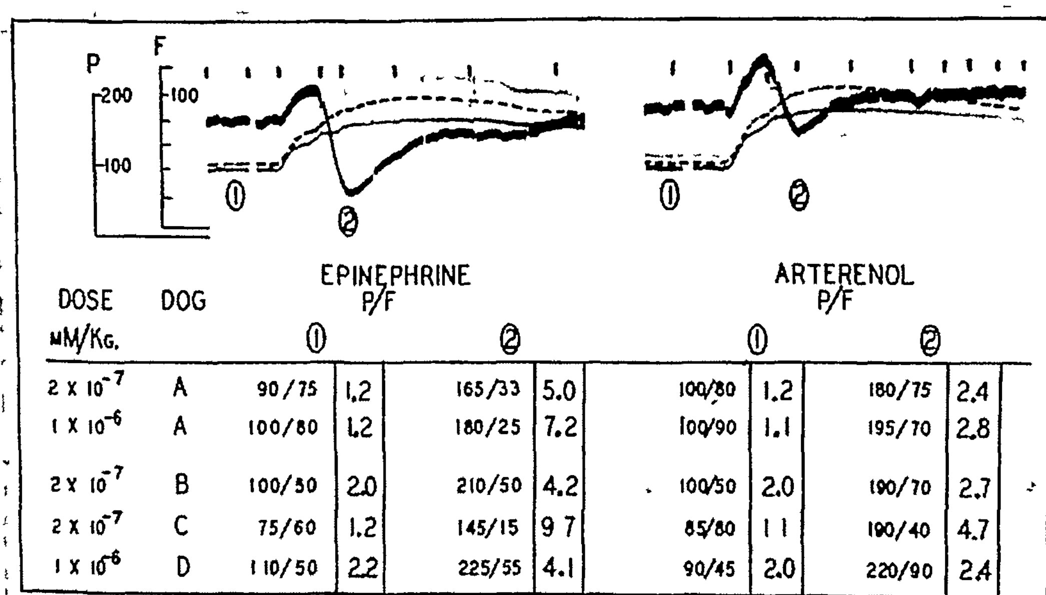
TABLE XII

Summary of the Relative Order of Activity of the Amines

Receptor	Order of Activity: Most active →					→ Least active
Vasoconstrictor	l-epi	dl-epi.	art	methyl-art	methyl-epi.	N-iso-art.
Uterine excitatory .	l-epi	dl-epi	art	methyl-art.	methyl-epi.	N-iso-art
Nictitating membrane excitatory .	l-epi	dl-epi.	art.	methyl-art	methyl-epi.	N-iso-art.
Dilator pupillae excitatory . .	l-epi	dl-epi	art	methyl-art	methyl-epi	N-iso-art
Ureteral excitatory	l-epi	dl-epi	art.	methyl-art	methyl-epi.	N-iso-art.
Intestinal inhibitory	l-epi.	dl-epi	art	methyl-art	methyl-epi	N-iso-art.
Vasodilator .	N-iso-art	l-epi.	methyl-epi	dl-epi	methyl-art	art
Uterine inhibitory	N-iso-art.	l-epi	methyl-epi	dl-epi	methyl-art.	art
Myocardial excitatory	N-iso-art	l-epi	methyl-epi	dl-epi	methyl-art	art

Reprinted, by permission, from Ahlquist, R P A study of the adrenotropic receptors. *Am J Physiol* **153**, 586 *(1948)*

very easy to see, the spleen contracts until it is obvious that it cannot get any smaller. Therefore, the 100 per cent mark is easy to determine The exact comparison between the epinephrine and arterenol depends on where they happen to be on the slopes Near the bottom of the slopes, the epinephrine is about five times more active than arterenol A little farther up on the slopes, it is ten times more active In the region where maximal contraction with epinephrine is obtained, the epinephrine is infinitely more active than arterenol In fact, I have not yet obtained a maximal contraction of the spleen with intravenous arterenol



DOSE μM/Kg.	DOG	EPINEPHRINE P/F ①		EPINEPHRINE P/F ②		ARTERENOL P/F ①		ARTERENOL P/F ②	
2 x 10⁻⁷	A	90/75	1.2	165/33	5.0	100/80	1.2	180/75	2.4
1 x 10⁻⁶	A	100/80	1.2	180/25	7.2	100/90	1.1	195/70	2.8
2 x 10⁻⁷	B	100/50	2.0	210/50	4.2	100/50	2.0	190/70	2.7
2 x 10⁻⁷	C	75/60	1.2	145/15	9 7	85/80	1 1	190/40	4.7
1 x 10⁻⁶	D	110/50	2.2	225/55	4.1	90/45	2.0	220/90	2.4

FIGURE 35 The comparative effects of intravenous epinephrine and arterenol on renal blood flow and resistance For explanation see text

Figure 35 shows a similar study, but one done on renal blood flow The records at the top of the Figure are examples of the actual records, but they have been clarified to make a better picture The dotted line represents the approximate mean renal arterial pressure (mm Hg) as determined from an optical record, which can be seen faintly under the dotted line The next line up is the renal arterial flow (ml per min), and those top little marks are urine drops as collected from the renal pelvis It can be seen that there is a difference in the shape of the flow curves, epinephrine is to the left and an equimolar dose of arterenol to the right

Shorr What is the time relationship?

Ahlquist There are faint breaks on the pressure record at ten second intervals, about eighty seconds of the record are illustrated

The usual one is to put the spleen in an oncometer and measure the volume changes. In the present method, the spleen is exteriorized through a midline incision in the abdomen and the spleen laid on a plastic plate. One edge of the spleen is fixed to the plate, a string from the other edge runs to a lever As the spleen contracts, one gets a record as from any other smooth muscle The dose of epinephrine or arterenol administered is based on what we call a "standard dose," which is 0 01 ml of M/1000 solution per kilogram. Several epinephrine and arterenol doses are illustrated to show that there is a difference in the response. With the arterenol doses, the arterial pressure rises higher, which might prevent the spleen from contracting However, we have studied enough dogs to know that by this method the size of the spleen is more or less independent of the arterial pressure

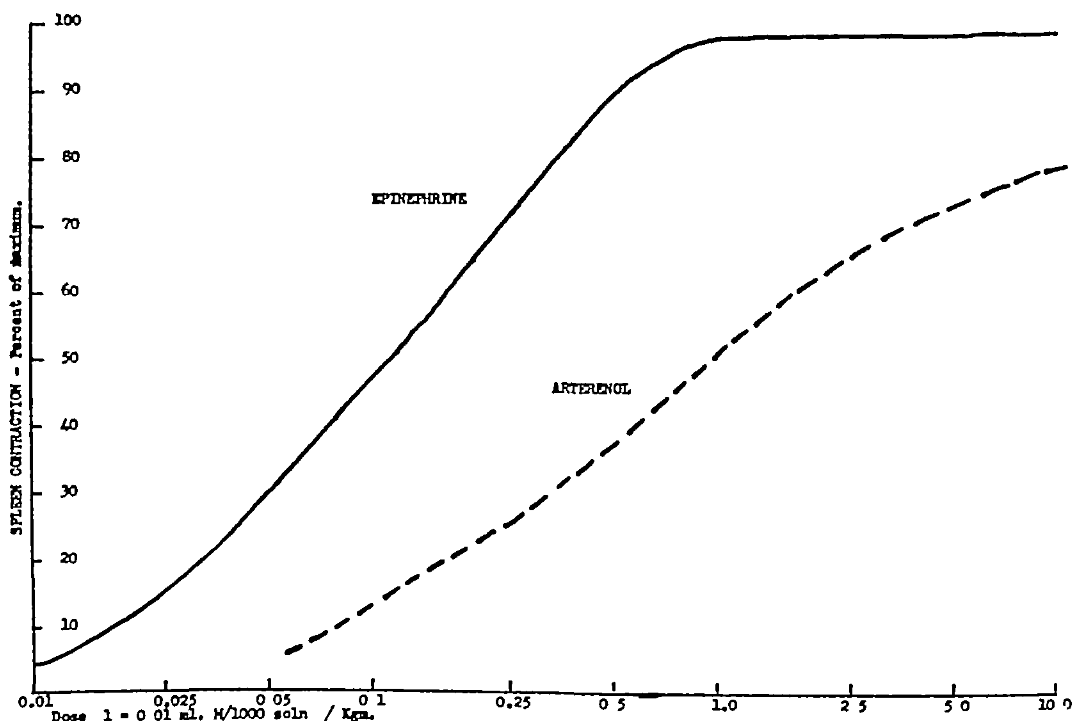
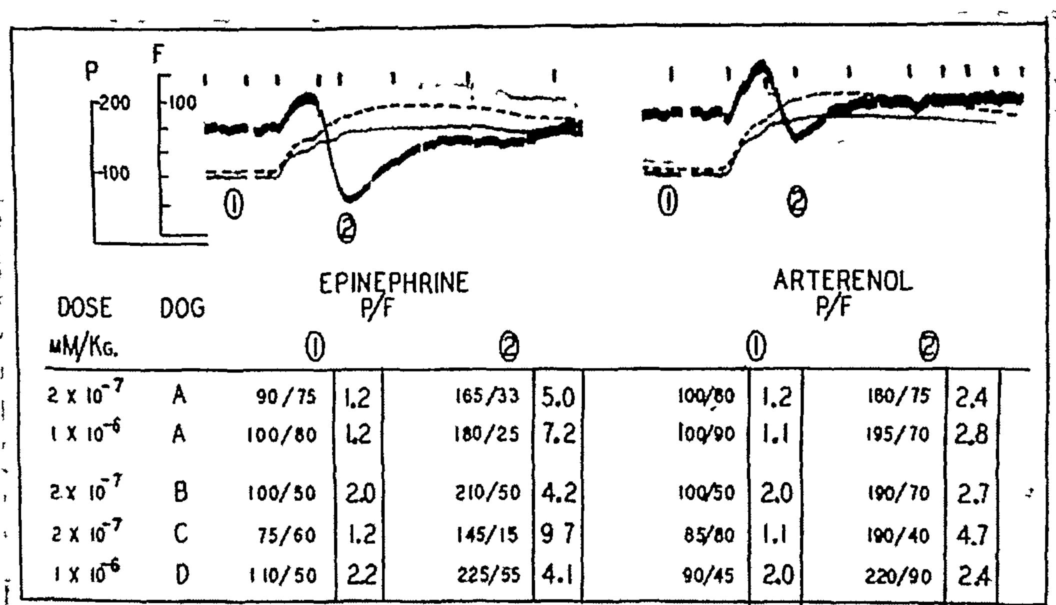


FIGURE 34 The dose-effect comparisons of epinephrine and arterenol on the spleen Average results obtained from six dogs

Figure 34 is a preliminary draft of the splenic dose-effect comparison in several animals The dosage is on the abscissae on a logarithmic scale, the 1 0 indicates the standard dose, as just mentioned The spleen contraction is expressed as per cent of the maximum contraction of that spleen The maximal contraction is

very easy to see, the spleen contracts until it is obvious that it cannot get any smaller Therefore, the 100 per cent mark is easy to determine The exact comparison between the epinephrine and arterenol depends on where they happen to be on the slopes Near the bottom of the slopes, the epinephrine is about five times more active than arterenol A little farther up on the slopes, it is ten times more active In the region where maximal contraction with epinephrine is obtained, the epinephrine is infinitely more active than arterenol In fact, I have not yet obtained a maximal contraction of the spleen with intravenous arterenol



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2×10^{-7}	A	90/75	1.2	165/33	5.0	100/80	1.2	180/75	2.4
1×10^{-6}	A	100/80	1.2	180/25	7.2	100/90	1.1	195/70	2.8
2×10^{-7}	B	100/50	2.0	210/50	4.2	100/50	2.0	190/70	2.7
2×10^{-7}	C	75/60	1.2	145/15	9 7	85/80	1.1	190/40	4.7
1×10^{-6}	D	110/50	2.2	225/55	4.1	90/45	2.0	220/90	2.4

FIGURE 35 The comparative effects of intravenous epinephrine and arterenol on renal blood flow and resistance. For explanation see text

Figure 35 shows a similar study, but one done on renal blood flow The records at the top of the Figure are examples of the actual records, but they have been clarified to make a better picture The dotted line represents the approximate mean renal arterial pressure (mm Hg) as determined from an optical record, which can be seen faintly under the dotted line The next line up is the renal arterial flow (ml per min), and those top little marks are urine drops as collected from the renal pelvis It can be seen that there is a difference in the shape of the flow curves, epinephrine is to the left and an equimolar dose of arterenol to the right

Shorr What is the time relationship?

Ahlquist There are faint breaks on the pressure record at ten second intervals, about eighty seconds of the record are illustrated

Below the records is a table giving the intravenous dose administered, the dogs used in the experiments, the pressure and flow values (first column) for the control periods① and for the periods of minimum flow②, and the peripheral resistance (second column) at these points. The peripheral resistance is the ratio of the pressure to the flow. The flow first increases, due to the rise in pressure since the flowmeter is in the renal artery. The flow increases until the drug gets through the flow meter and into the kidney, then the renal constriction occurs In all cases, comparing equimolar doses, the epinephrine is a much more potent renal constrictor than arterenol. This is also apparent in the urine flow. The urine output is diminished much more with the epinephrine than with the arterenol

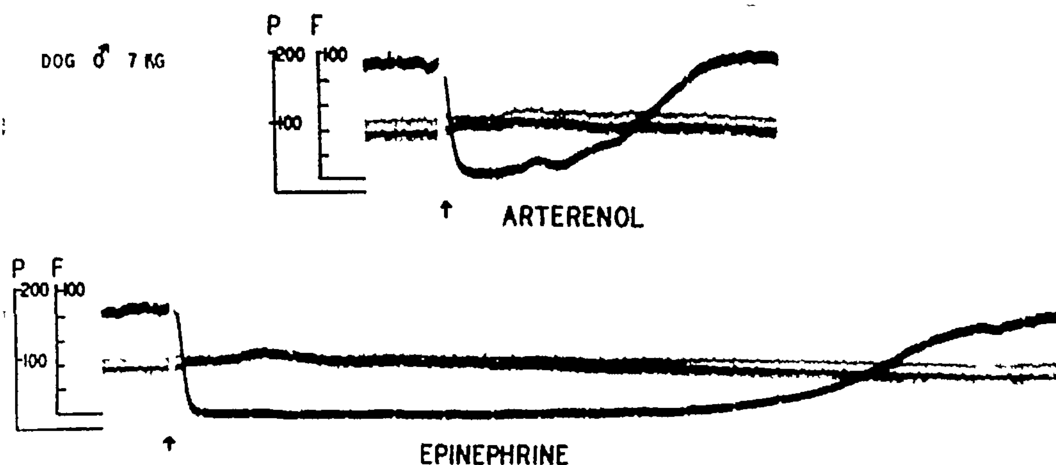


FIGURE 36 The comparative effects of intra-arterial epinephrine and arterenol on renal blood flow Pressure (P) in mm Hg, Flow (F) in ml/min At the arrows, equimolar doses administered

Figure 36 shows what happens if the drugs are injected into the renal artery. These are equimolar doses, equivalent to about one microgram Since these are injected into the renal artery, the vasoconstriction occurs at once With arterenol, the flow drops to zero, then returns to normal within a period of fifty seconds With the same dose of epinephrine, the flow drops to zero, and stays there. With the rotameter, one is never sure about the lower one or two milliliters per minute flow, but as far as one can tell, it is approximately zero Although we were not recording the urine flow in this animal, there would be no urine produced during the period of constriction

When one examines all of the data about the comparative effectiveness of epinephrine and arterenol, it becomes obvious that

there is a general rule relating the potency of the two compounds. That general rule is. Epinephrine is more potent than arterenol. The principal exceptions to this rule at the present time are the comparative actions on the heart and intestine in various species. The human heart, as shown by the rate and output, presumably follows the rule quite well However, in the open-chest dogs, arterenol is apparently more active than epinephrine. On some perfused heart preparations, arterenol is presumably more active. It is very difficult to determine whether these are real exceptions. If I had time, I could go into some of the criticisms of the usual perfused heart preparations in which it can be shown that there are physical reasons why arterenol usually appears to be more potent than epinephrine when, in actuality, it may not be

On the rat colon, work which has been published by Gaddum (65), and others, shows that arterenol is sometimes more active than epinephrine in relaxing this structure However, their results are open to question, not as to what occurs, but as to their *in vivo* significance. These workers used a low calcium bath at a low temperature, and determined the spasmolytic potency of the amine against acetylcholine On the gut in general, especially *in vivo* in the dog, rabbit, or cat, which are the three I have used, epinephrine is more active than arterenol, but not much more active

The chief question that I should like to raise in your minds is this. Since epinephrine is more potent than arterenol, is arterenol (which is found in the greater amount) or epinephrine (which is found in the lesser amount) the mediator? Take the spleen, for example On the spleen, arterenol has only about ten per cent of the activity of epinephrine According to Dr von Euler's work, in the splenic nerve there is about ten per cent, or less, of epinephrine Therefore, according to a rough calculation, the same response is found whether it is the 90 per cent arterenol which is contracting the spleen, or whether it is the 10 per cent epinephrine in the nerve which is contracting the spleen. I feel that until we have more information we cannot accept without question the proposal that nor-epinephrine is the mediator, because it is perfectly possible that the trace of epinephrine — in some cases it is not a trace, it is a significant amount — is the mediator

This brings us to the main anatomical site which has been questioned the neuro-effector junction. What goes on at the nerve ending? Does epinephrine (or arterenol) come from the nerve end and act on the muscle? Do they act in such a way that the exact mediator or mechanism cannot be determined?

von Euler: In the splenic nerve, the epinephrine content is extremely low. I said that in nerves in general it would not exceed 5 to 10 per cent. As a matter of fact, in the splenic nerve our figures have been around 2 per cent.

Ahlquist: Yet, as shown in Figure 34, you cannot get a maximal splenic contraction artificially with nor-epinephrine, but you can do it easily with epinephrine. So we still come back to the question of what really happens in this place that we cannot get at. the nerve ending, the neuro-effector junction?

EDITOR'S NOTE: Dr Ahlquist had the following comment to add after the Conference.

> Dr. von Euler has shown that, while the splenic nerve contains not more than about 3 per cent epinephrine, the spleen itself may contain up to 20 per cent It is perhaps reasonable, therefore, to assume that the concentration of epinephrine at the nerve ending could be about 10 per cent.

Nickerson: It is possible that the adrenergic mediator is not the same at all loci. Because of the limitations of the available techniques, we speak of mixtures of epinephrine and nor-epinephrine in nerves and tissues without really implying whether these reflect mixtures of mediators within nerve fibers, or mixtures of nerve fibers with different mediators It is only a straw in the wind with respect to this question, but I was quite impressed by a paper by Downman in a recent issue of the *Journal of Physiology* (66), in which he showed rather nicely that the areas of intestinal relaxation, and the areas of vasoconstriction, are not the same following stimulation of a single mesenteric nerve bundle In other words, the nerve fibers coming in through one of the arcades produce relaxation in an area somewhat different from that in which they produce vasoconstriction This indicates rather directly that the fibers subserving these two functions are different The mediator may also be different

Selkurt. There is another interesting phenomenon that could be introduced here, I think, and that is the increased sensitivity to the action of these drugs when the organ is denervated I wonder if it might not be appropriate to introduce at least one Figure to show the difference in response of the normal kidney, and the organ which is denervated This is work done by Dr Robert M Berne (67) of our department

Figure 37 is a group summary of about 13 dogs showing the means of the responses under anesthesia Most of the animals were under pentobarbital anesthesia, but a few were under chloralose This Figure compares commercial epinephrine, *l*-epinephrine and

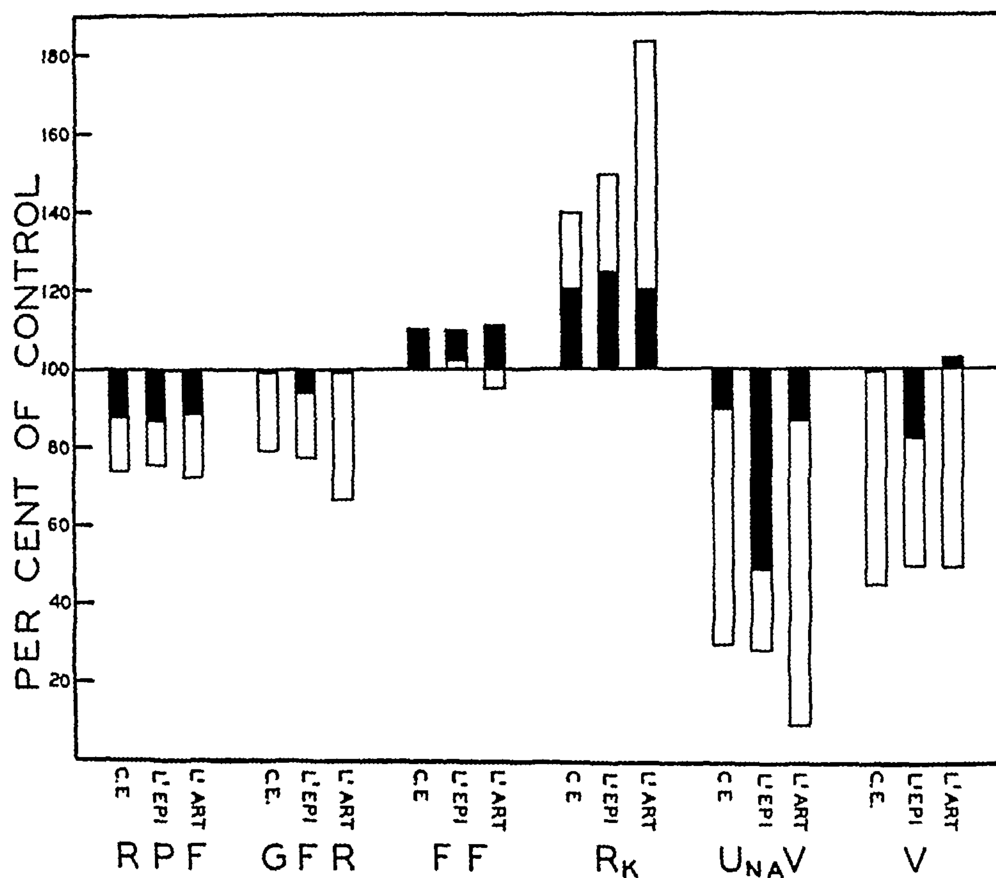


FIGURE 37 Showing the effects of commercial epinephrine (C E), *l*-epinephrine and *l*-arterenol on renal function Solid bars nerves intact, hollow bars denervated kidney RPF renal plasma flow (PAH clearance); GFR glomerular filtration rate (creatinine clearance), FF filtration fraction, (ratio of creatinine over PAH clearance) R_K renal resistance, $U_{Na}V$ urinary excretion of sodium, V urine volume

nor-epinephrine in terms of per cent deviations from the control for R P F , the renal plasma flow, G F. R., the glomerular filtration rate, F F , the ratio of the latter to the former, the filtration fraction; and R_K, which is the total renal resistance. We have data on the breakdown of the renal vascular components, but this Figure gives only the total renal resistance In addition, there are, I think, some phenomena which are of interest because of the effect of these hormones on the excretion of sodium and urine volume. The solid columns represent the response of the innervated kidney. The open ones are the responses of the kidney which had been denervated. I might add that the clearances were done at intervals ranging from 9 to 45 days after the nerves had been completely removed from one kidney

To continue with what Dr Ahlquist has said, renal resistance goes up with the reduction of R P F In the normal structure,

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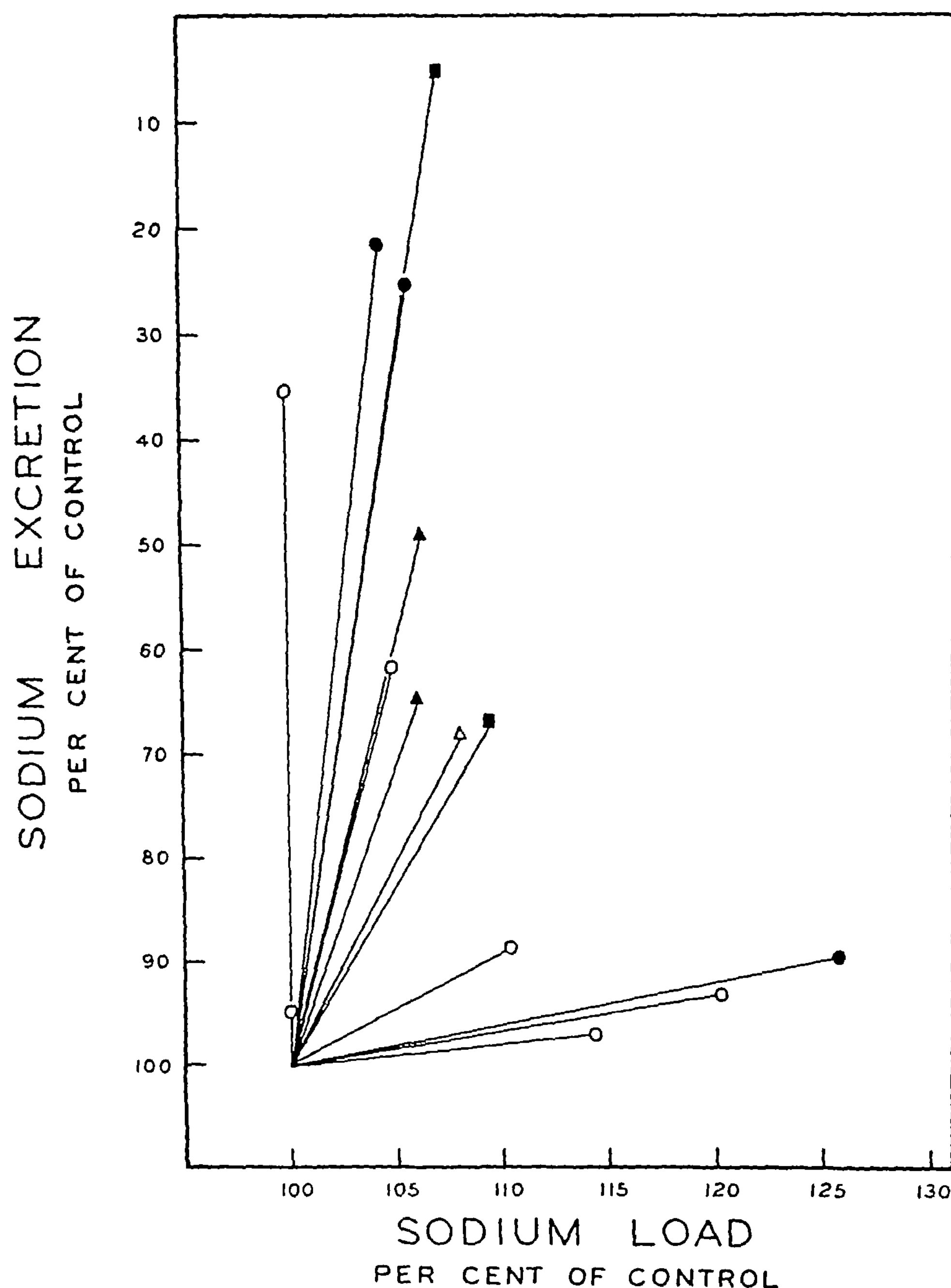


FIGURE 38 Depression of sodium excretion by *l*-epinephrine (circles), by *l*-nor-epinephrine (triangles), and commercial epinephrine (squares) in the face of increase in tubular load in the normal (open symbols) and denervated (solid symbols) kidneys Reprinted, by permission, from Berne, R M, Hoffman, W K, Jr, Kagan, A, and Levy, M N Response of the normal denervated kidney to *l*-epinephrine and *l*-nor-epinephrine *Am J Physiol* **171**, *564 (1952)*

the sodium excretion, which is along the ordinate It includes all the experiments in the entire series in which there were increases in load, or at least no decrease in load, since the renal physiologists

increased renal resistance is explicitly in favor of epinephrine, although I don't think the differences are as great as his data show However, one is struck with the fact that the kidney without the nerves responds to a much greater extent, and under these circumstances, apparently, arterenol creates the greater response. The constriction is greatest in the denervated kidney in response to arterenol.

With regard to the other data, I don't think they are particularly relevant, except that they show on the average a fall in filtration rate that is related to the vasoconstriction That, of course, is greater in the denervated than in the normal kidney.

The excretion of sodium is particularly interesting to us because it indicates another function of the hormones that has been touched on, i e, the problem of hormonal influence on tubular function, aside from hemodynamic changes. Indeed, the excretion of sodium may be depressed even while the sodium load (product of plasma sodium concentration times the glomerular filtration rate) stays the same or goes up, so that there is enhanced tubular reabsorption of sodium as a more or less specific response to the action of these particular hormones As can be seen, it goes on in connection with all three of these types of preparations The effect is more marked in the denervated kidney, where it is greater in response to arterenol The urine volume roughly parallels the change in sodium excretion, and our feeling is that it follows on an obligatory osmotic basis the alteration in sodium excretion The thing that I wanted to emphasize is, of course, the effect of denervation, which most strikingly enhances the effect of these drugs I might add, too, that the hormones were given by continuous infusion in doses of 1 to 2 μg per minute per kilogram, which is a physiologist's dose and is somewhat high, I think, compared to what we have heard about

Burton Would you interpret these changes as another case of Cannon's sensitization idea?

Selkurt. Yes, it is a sensitization phenomenon But the interesting thing is that the sensitivity not only extends to vascular phenomena but to the problem of sodium reabsorption, which I think is worth considering

Moe Are you certain, Dr Selkurt, that these changes in sodium and water excretions could not possibly be due to a redistribution of blood flow?

Selkurt One thing I can say is that it is not due to reduced blood flow Figure 38 relates to sodium load, which is given along the abscissa in terms of per cent of the control, as compared with

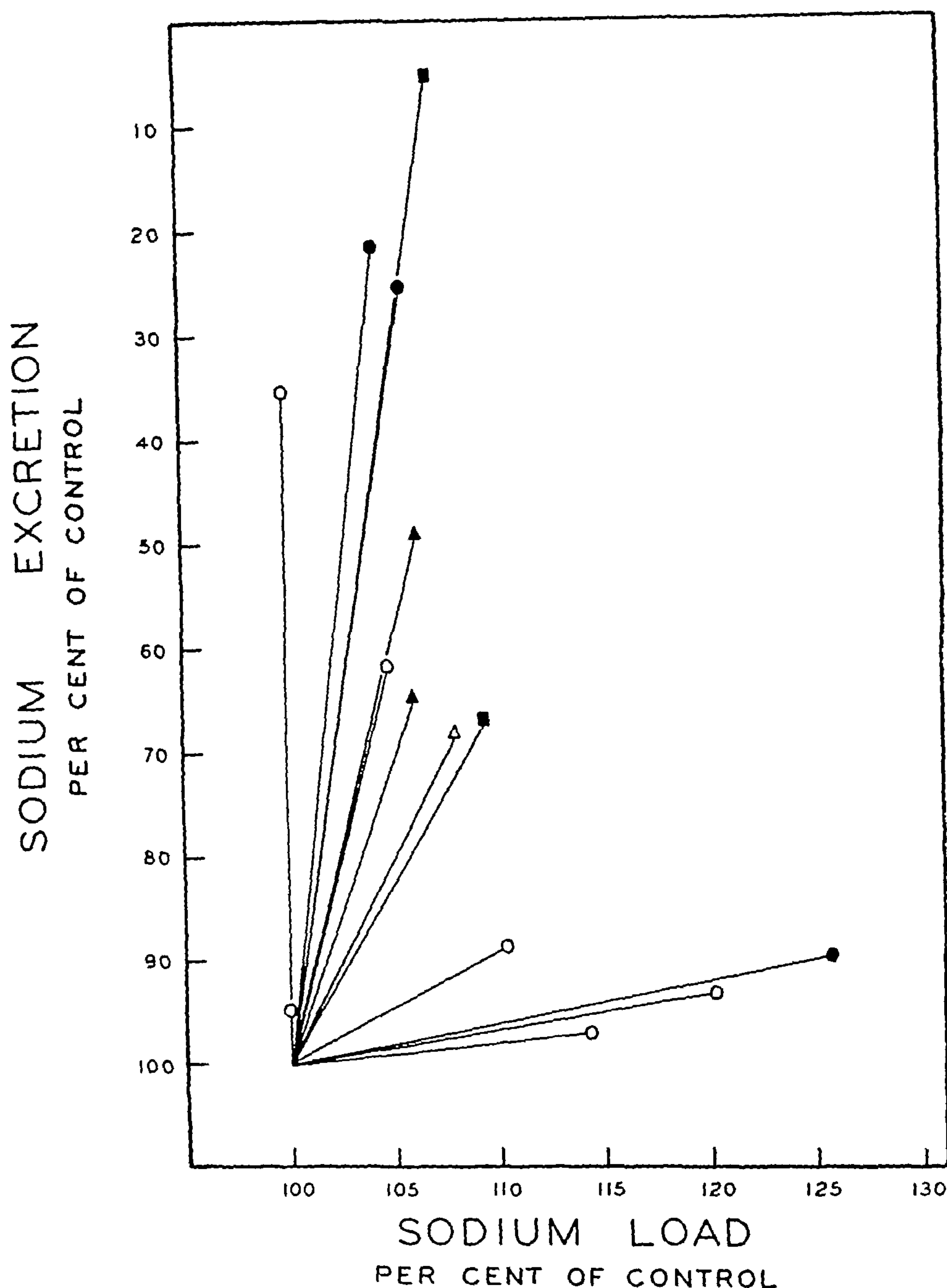


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the sodium excretion, which is along the ordinate. It includes all the experiments in the entire series in which there were increases in load, or at least no decrease in load, since the renal physiologists

are aware of the fact that reduced load may be an important factor in modifying sodium reabsorption. The line with maximum slope represents a group of about a dozen experiments in which these drops in excretion occurred, with either small increases in load or no change.

I think the important problem here, in terms of the electrolyte phenomena, is whether the load is reduced If we can rule that out, I think we can safely say it is probably a tubular phenomenon.

Ussing. We have been working with the isolated frog skin, studying the active transport of sodium inward which this organ performs The frog skin responds violently to epinephrine The resistance to sodium movement drops At the same time, what we call the electromotive force of the sodium transport drops, too That is, the force available to drive sodium through decreases In so far as organs like the tubule and the frog skin can be compared, that would be in accord with the view that there could be a direct effect of epinephrine upon sodium reabsorption

Nickerson. Do I interpret your data correctly as indicating, at least in the denervated kidney, that nor-epinephrine rather than epinephrine is the more potent with respect to what we must consider to be a metabolic function?

Selkurt. That is right I might add that actually, in terms of over-all renal resistance, it appears (67) that nor-epinephrine is more effective than epinephrine in increasing that resistance in the denervated kidney In the innervated kidney they seem to be about equal In other words it seems to be true, in a sense, not only for the sodium mechanism in both groups of dogs, but for the vascular. That difference is not so great in the normal kidney

Burch. I should like to ask Dr Ussing if his studies on the frog skin indicate that these two agents might interfere with the amount of sodium that filters through, assuming, of course, that the rate of filtration of sodium is essentially correct as indicated

Selkurt It would be a question of whether it would interfere with the diffusion of sodium through the membranes But if our filtration rate is right, we must assume that sodium will pass equally quickly.

Ussing. Yes, that is right

Stead. I should like to ask if there was a reduction in the excretion of sodium at the time that the blood flow was measured

Selkurt. Yes I am not resting my argument so much on the alteration in blood flow Your question implies that even though

filtration rate was maintained, there was some continuous reduction in flow that might have had a bearing on sodium excretion.

Stead: Yes.

Selkurt: The over-all data that apply to the filtration fraction showed that it went up in the kidneys with intact nerves, which indicated that there was a more marked effect on plasma flow than on filtration rate This would tend to answer your question in the affirmative, although I don't know what bearing it would have on the urine flow In other words, in these experiments the plasma flow is apparently more reduced than filtration, but the crux of the argument concerning the relationship of tubular reabsorption to the load is not changed. If total blood flow depreciation has an influence on sodium reabsorption, I don't know offhand what this influence might be.

Stead: I just wonder whether the speed of blood flow surrounding the tubules has any effect on the composition of the urine I think it is a fair question to ask, I don't know what the answer is

Knisely May I ask a question which may affect some of the interpretations? Two general classes of chemical reactions may be considered, one stoichiometric reaction In such reactions two molecules come together and react with each other forming, let us say, a precipitate The other we might call enzymatic reactions It is conceivable that one class of molecule in the system of reaction may be used over and over again.

Suppose that a molecule of, say, epinephrine or nor-epinephrine were used up stoichiometrically at the junction of, say, a nerve ending and a muscle If the reaction were stoichiometric, the molecule of free epinephrine, or nor-epinephrine, would not then reappear in the blood down stream from that myoneural junction

If, on the other hand, the molecule of epinephrine or nor-epinephrine operated as a part of some enzyme system, then the molecule could carry out its physiological function at the myoneural junction and be released, and would appear later down stream in blood coming from that region

Is there any information today which would settle the question of whether we are measuring simply those molecules not being used up in the reaction at the myoneural junction, or whether we are measuring molecules which participate in reactions and are then released?

von Euler I don't think there is any specific knowledge on that subject, but I think one might imagine that the molecules found, so to speak, in the blood stream, are those which have escaped

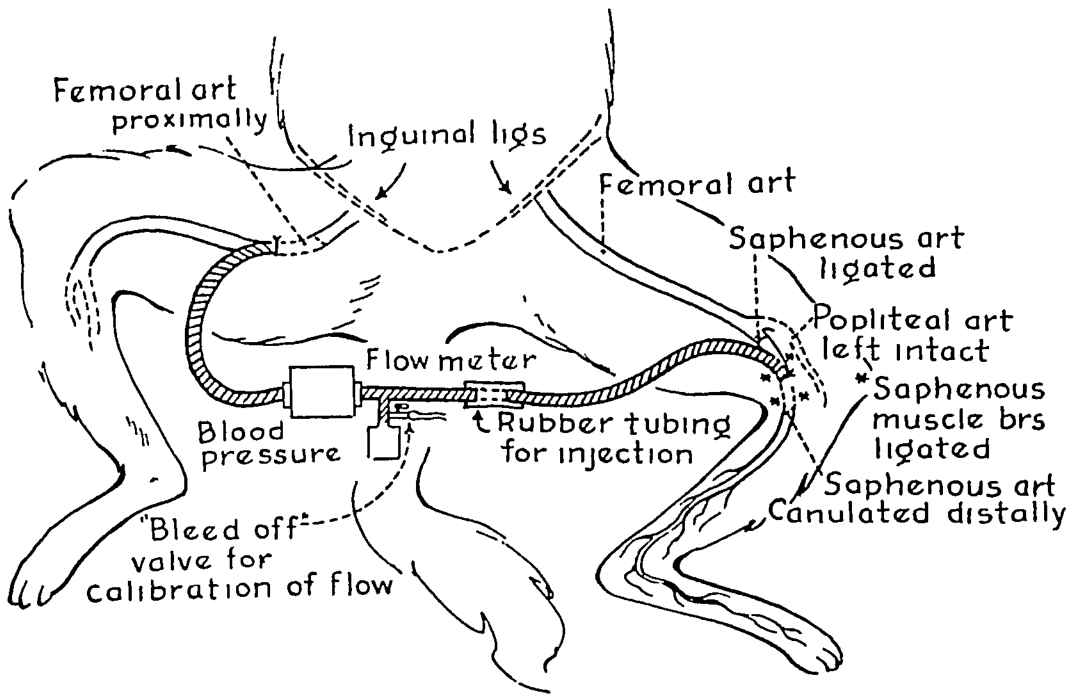
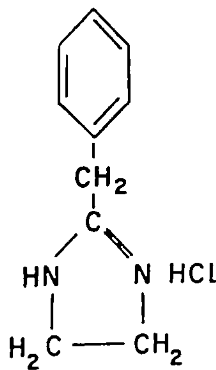
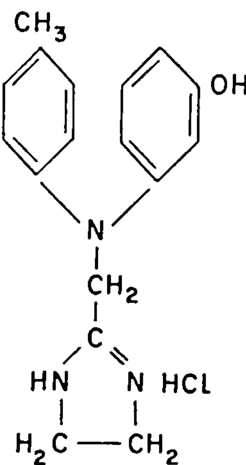


FIGURE 40 Technique for flow measurement in skin





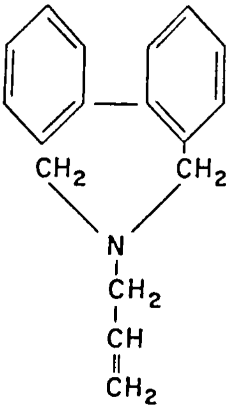


FIGURE 41 Structural formulae for the three antiadrenergic drugs used in the study Priscoline and Regitine are supplied by Ciba Pharmaceutical Products, Inc , Summit, N J , Ilidar is supplied by Hoffmann-La Roche, Inc , Nutley 10, N J

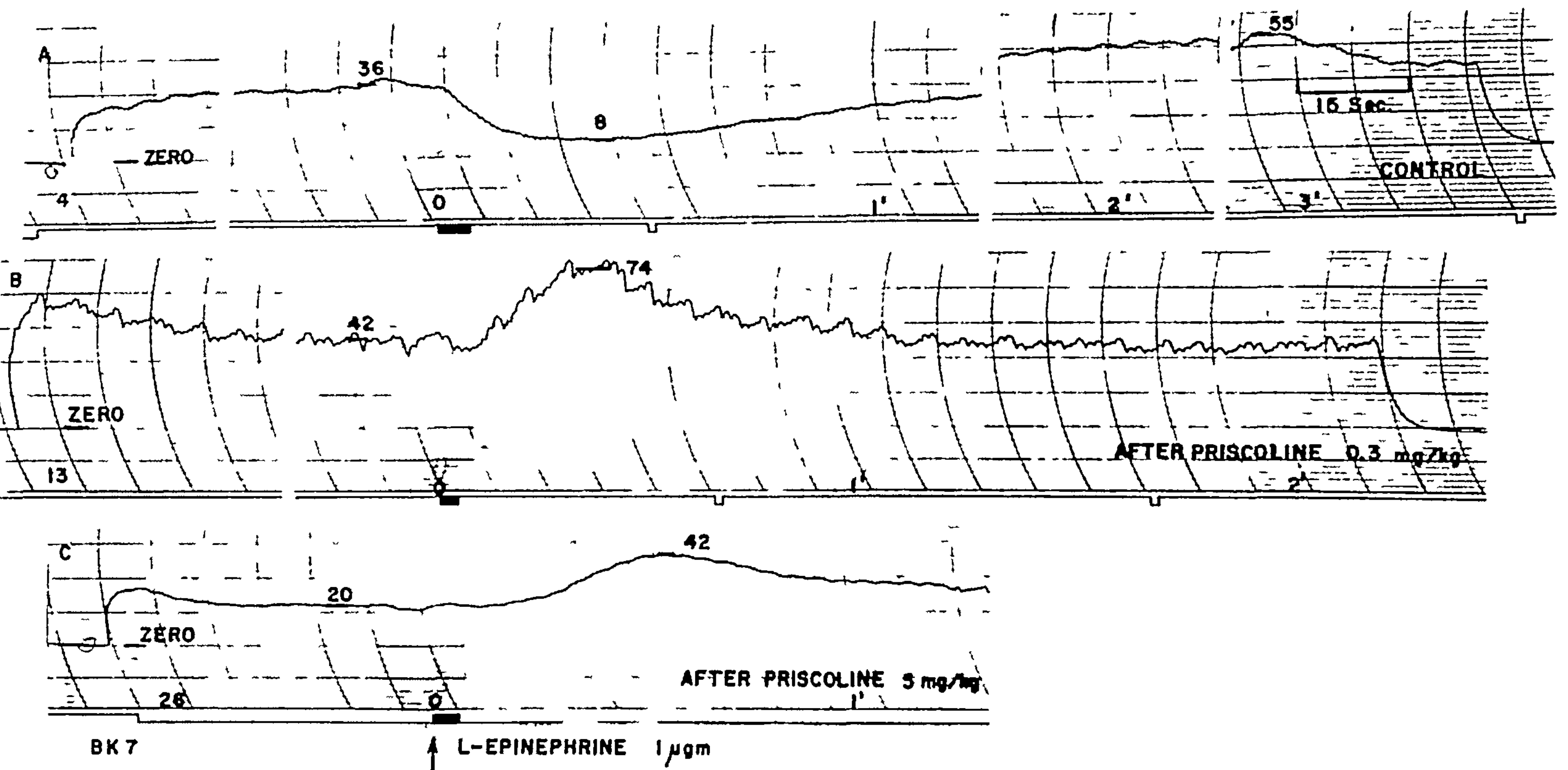


FIGURE 42 Flow responses to 1 μgm intra-arterial injections of *l*-epinephrine in a vascular bed supplying skeletal muscle before (A) and after (B, C) intra-arterial injections of the antiadrenergic drug Priscoline Figures adjacent to the curves give the rate of flow in ml/min ZERO indicates the position of the recording line at zero flow, usually the second heavy line up from the bottom Numbers in lower left hand corners of records (4, 13, 26) give the serial number of the injections, and the number in the lower corner of the figure (BK7) is the experiment number Time is 7 5 seconds between vertical arcs The signal magnet gives the interval of the epinephrine injection The figures near the bottom of the record (0, 1′, 2′, etc) give the time elapsing in minutes from the beginning of the injection The segments of zero flow at the beginning and ending of the records are checks on the zero flow position of the records and are included to demonstrate the small degree of instrumental drift Note primary dilator response in records B and C and secondary dilator response in record A These may be compared with the dilator response to ischemia noted just after the first zero flow check at the left end of the records

cessively increasing doses of one of the adrenergic blocking drugs. The antiadrenergic drugs used in this study were Priscoline, Regitine (C-7337) and Ilidai (RO2-3248) (see Figure 41) The latter is structurally quite similar to Dibenamine, except that it is water soluble and its action is more rapid in onset, and of shorter duration

Figure 42 shows the normal constrictor response in muscle to the injection of *l*-epinephrine After an appropriate dose of the antiadrenergic drug, a pure and marked vasodilator response follows this dose of epinephrine If the dose of the antiadrenergic drug is increased to very large doses, eventually the dilator response can be blocked, however, the dose of the blocking drug must be increased to about 100 times the epinephrine reversing dose to produce this effect

Figure 43 shows the response in skin to the same dose of epinephrine and to doses of Priscoline comparable to those used in muscle Epinephrine per se produced a vasoconstriction in skin similar to

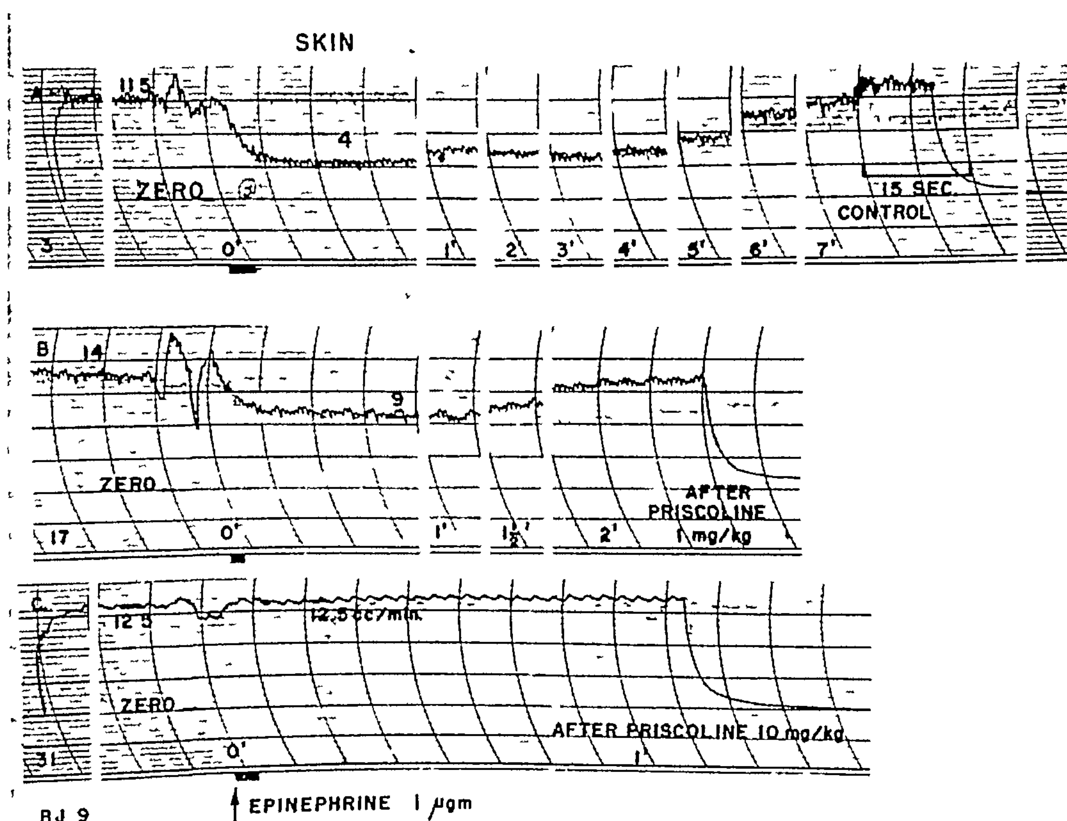


FIGURE 43 Flow responses to 1 μgm intra-arterial injections of *l*-epinephrine in a vascular bed supplying skin before (A) and again after (B, C) intra-arterial injections of priscoline For further description see legend for Figure 43 Note absence of dilator responses The upward and downward movements of the lines at 0′ time are arti facts introduced by the injections

that noted in muscle. When increasing doses of the blocking drug were given, there was simply a progressive decrease in constriction with no vasodilation, either primary or secondary These differences in response represent a fundamental difference between these two vascular beds (69,70)

Moe. I might say, before you go on, that the kidney is much like the skin in this respect

Green With *l*-nor-epinephrine in muscle, a typical constriction was again found (Figure 44) In a few of the animals, there was a secondary vasodilation such as was obtained with *l*-epinephrine When the blocking drug was given, however, no conversion of the response to vasodilation was noted, the only change was a progressive decrease in the degree of constriction

Stead What was the time on that?

Green The times are marked by the vertical arcs. 7 5 seconds elapse between each arc Minute intervals are marked by the signal at the bottom and are indicated by the figures near the bottom of the record

Stead· Could that vasodilation be maintained in the muscle?

Green· I think with either a continuous infusion, or with a larger dose of epinephrine, it could be, the larger doses after the blocking drugs cause quite prolonged vasodilation in dogs

Stead I mean before the block

Green. Are you referring to the secondary dilation?

Stead Yes

Green I don't know If the drug is given intravenously, there may be temporary or small early dilation, followed by a prolonged vasoconstriction

Moe Are you comparing epinephrine and nor-epinephrine in hyperreactors and hyporeactors?

Green In about half of the experiments, there was a secondary phase of dilation after the initial constrictor response to epinephrine and before administration of the blocking drug We classified the muscle experiments into hyperreactors in the presence of a secondary dilator response to epinephrine, and hyporeactors in its absence

Moe This is no more than what might be expected after a period of one minute of almost zero flow?

Green The vasodilation that follows a comparable period of reduced flow, produced by mechanical compression at the blood vessel, is usually much less in magnitude, and shorter in duration, than that which follows an intra-arterial injection of epinephrine

cessively increasing doses of one of the adrenergic blocking drugs. The antiadrenergic drugs used in this study were Priscoline, Regitine (C-7337) and Ilidar (RO2-3248) (see Figure 41) The latter is structurally quite similar to Dibenamine, except that it is water soluble and its action is more rapid in onset, and of shorter duration

Figure 42 shows the normal constrictor response in muscle to the injection of *l*-epinephrine After an appropriate dose of the antiadrenergic drug, a pure and marked vasodilator response follows this dose of epinephrine If the dose of the antiadrenergic drug is increased to very large doses, eventually the dilator response can be blocked, however, the dose of the blocking drug must be increased to about 100 times the epinephrine reversing dose to produce this effect.

Figure 43 shows the response in skin to the same dose of epinephrine and to doses of Priscoline comparable to those used in muscle Epinephrine per se produced a vasoconstriction in skin similar to

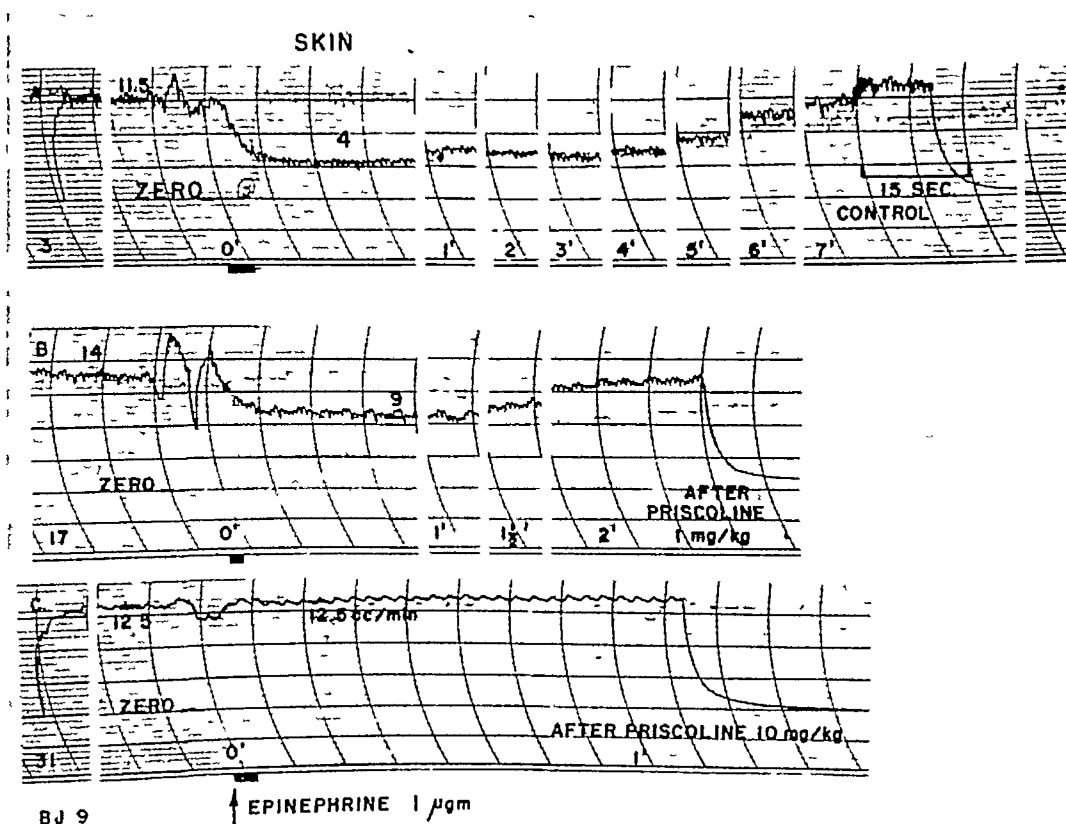


FIGURE 43 Flow responses to 1 μgm intra-arterial injections of *l*-epinephrine in a vascular bed supplying skin before (A) and again after (B, C) intra-arterial injections of priscoline For further description see legend for Figure 43 Note absence of dilator responses The upward and downward movements of the lines at 0′ time are arti facts introduced by the injections

It is probable that the secondary vasodilation does not represent reactive hyperemia (see Figures 39 and 40).

Moe Could it be a reactive hyperemia antagonized by nor-epinephrine still present in the system?

Green The secondary dilation is present only in about half of the animals, whereas the response to ischemia occurs in all muscle preparations

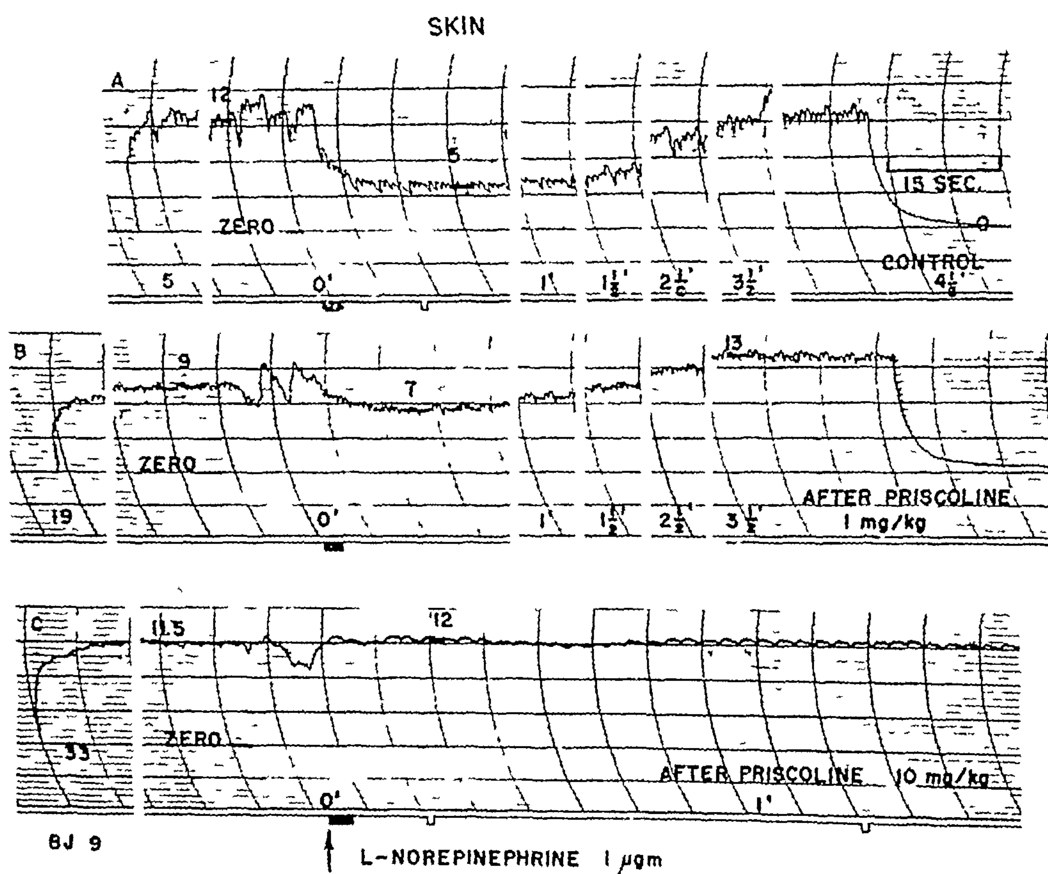


FIGURE 45 Flow responses in a cutaneous vascular bed to intra-arterial injections of 1 μgm of *l*-nor-epinephrine before and again after, intra-arterial injections of Priscoline Same experiment as Figure 44 See also legend to Figure 43 Note absence of primary dilator response

Figure 45 shows the response in skin to nor-epinephrine. The initial response is pure constriction The constriction is blocked by the increasing doses of the blocking drug with no conversion to a dilator phase

To summarize briefly, nor-epinephrine and neosynephrine, which I do not have time to discuss, both cause pure constriction in skin; this constriction is gradually abolished by the blocking drug In

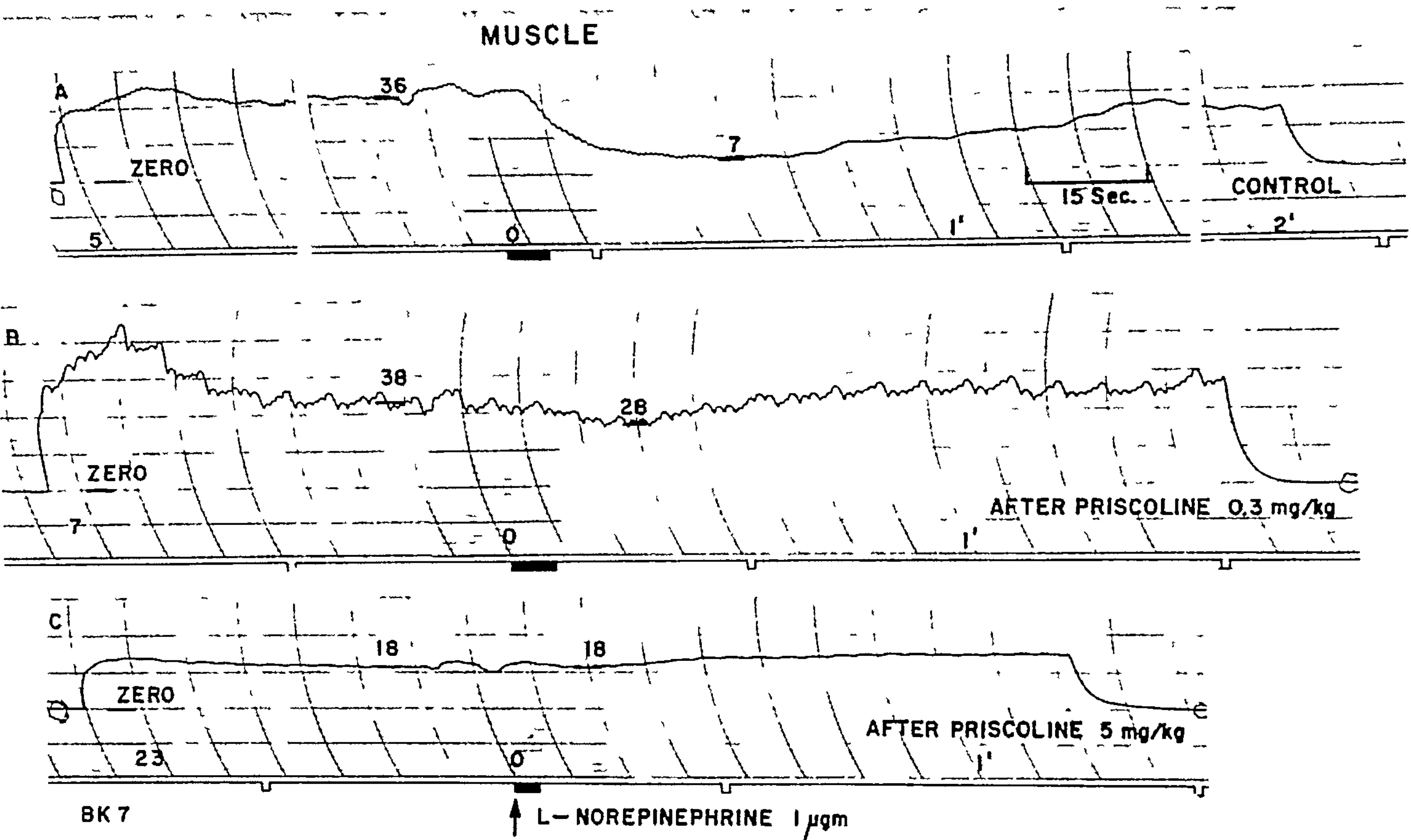


FIGURE 44 Flow responses in a muscle vascular bed to intra-arterial injection of 1 μgm of *l*-nor-epinephrine before, and again after, intra-arterial injections of priscoline See legend to Figure 43 for explanation. Same experiment as Figure 43 Note absence of dilator responses

mostly in terms of specificity and accuracy. These techniques measure not only the oxygen which replaces the substrate's amino group, but also the oxygen which is taken up by other parts of some of the substrates We therefore had to devise procedures which employ the determination of the ammonia evolved during the deamination

For most of the work which I shall discuss, we relied on the Conway microdiffusion methods In addition to being accurate and specific, these techniques are flexible in the matter of the sensitivity

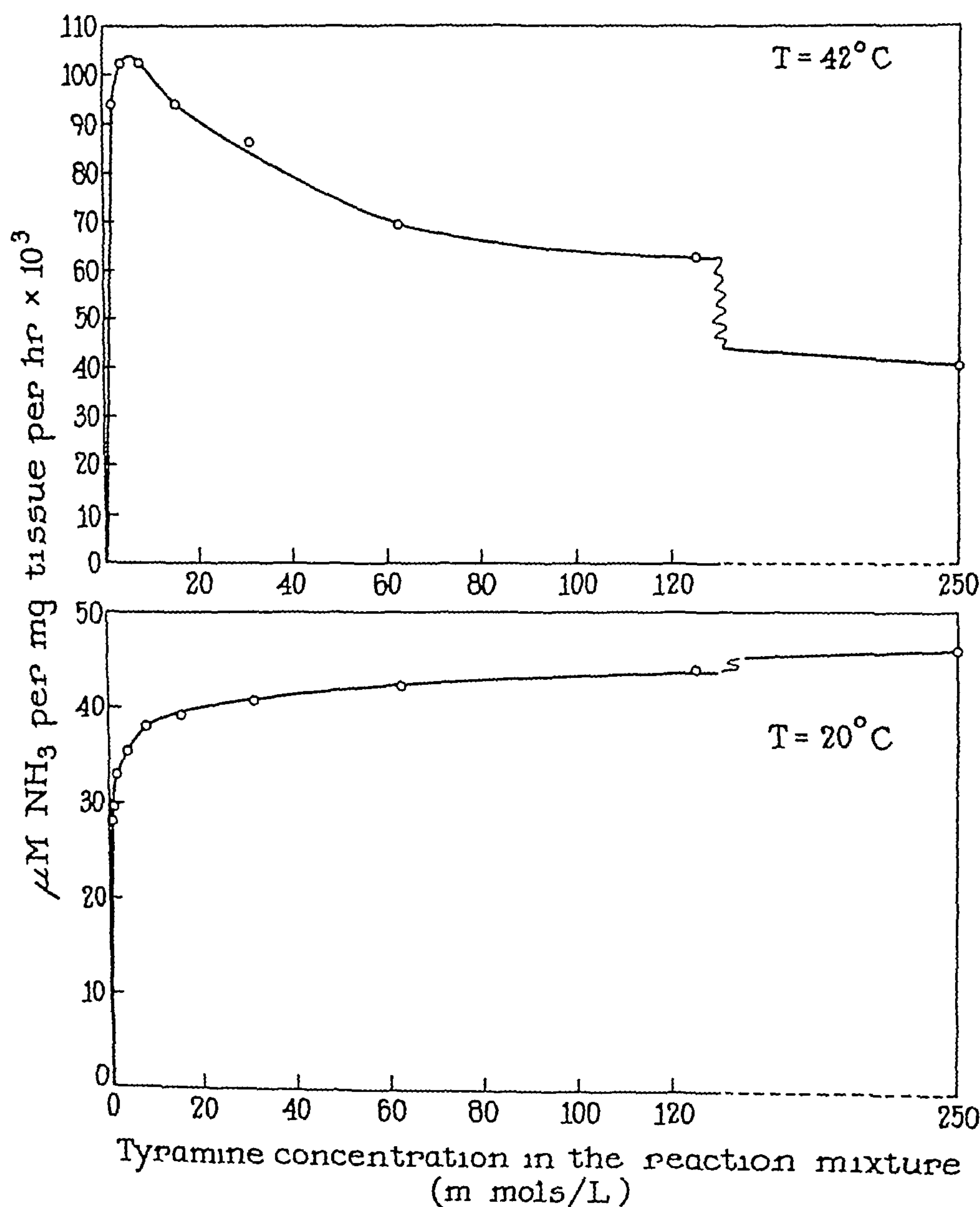


FIGURE 46

skin, *l*-epinephrine behaves likewise, but in muscle the epinephrine constriction is converted to a pure vasodilation with very small doses of the blocking drug. Nor-epinephrine and neosynephrine cause constriction in muscle which is gradually blocked as the anti-adrenergic drug is progressively increased

Ahlquist· Which is the more potent in skin?

Green. There is not a great deal of difference between the responses to 1 μg doses of *l*-epinephrine, or to 1 μg doses of *l*-nor-epinephrine in skin, or in muscle, before administration of the blocking drug

Ahlquist. I asked because there are a good number of other ways in which skin constriction has been tested, such as intracutaneously in humans with a local anesthetic, in rabbits with trypan blue, and in the dog by means of a photoelectric cell on the ear In all of them, epinephrine is from five to ten times more potent

Green Differences of the order of five or ten times are definitely not seen in our studies

Nickerson· Can the difference between your results and Dr Ahlquist's be related to dosage? The dose given by you may be fairly well up on the dose-response curve, where the difference between epinephrine and nor-epinephrine may be slight

Green· We haven't explored a range of doses of epinephrine in either the isolated skin or muscle preparations However, see references (71,72) for data on the whole leg

Nickerson The dose could make a big difference in the observations

MONO AMINE OXIDASE SUBSTRATES AND INHIBITORS

Shorr Just to keep us thinking after we leave the meeting, I should like to turn to another aspect that is, a consideration of some of the mechanisms through which amines may be dealt with by tissues on which they operate

Cotzias: I was keenly interested in the statements of Dr von Euler and Dr Goldenberg that a maximum of four per cent of the injected nor-epinephrine and epinephrine are recovered in the urine I shall try to point out a few of the things that might be happening to the other 96 per cent

Dr Vincent P Dole and I have been interested for quite a while in the enzyme mono amine oxidase, which oxidatively deaminates *in vitro* several amines Early in our work we found that the classical respirometric techniques, which we were using for the measurement of the enzyme's activity, were subject to several shortcomings,

tures, there is a wide range of substrate concentrations, the variation of which does not affect the performance of the enzyme Our working hypothesis on this phenomenon, which is not restricted only to tyramine, is that the amine promotes the thermal denatura-tion of this as well as other enzymes. Be that as it may, this situation forced us to adopt lower temperatures of incubation than those conventionally used in this type of work, so as to avoid a multiplicity of forces which otherwise would adversely affect the average reaction velocity of this enzyme system With these precautions, it is quite easy to determine accurately the activity of even small amounts of mono amine oxidase Furthermore, in the study of inhibitors, one can be more confident that the inhibition observed is due to the inhibitor, rather than to the substrate

TABLE XIII

Distribution of Mono Amine Oxidase

	Enzyme		Nitrogen		Specific Activity	
Fraction	Units / g wet tissue	% of homogenate	mM N / g wet tissue	% of homogenate	Units enzyme / mM N	% of homogenate
N	5 3±1 6	21 9	49± 18	23 1	11 7± 3 1	98± 17
NW	3 1±1 5	12 9	11± 06	5 2	33 ±18	312±218
M	13 7±1 8	56 8	56± 15	26 4	26 ± 6 9	219± 25
S	1 9±1 2	7 9	91± 33	42.9	2 6± 2	20± 14
Sum	23 8	99 5	2 07	97 6		
Homogen	24 1±2		2 12± 38		11 9± 2 6	

The fractions are those obtained by differential centrifugation in 0 88M sucrose The numbers are the means and standard deviation from 13 experiments

Reprinted, by permission, from Cotzias, G C , and Dole, V P Metabolism of amines II Mitochondrial localization of mono amine oxidase *Proc Soc Exper Biol & Med* **78,** *157 (1951).*

In order to convince ourselves further on the validity of this measurement, we performed the experiments summarized in Table XIII The symbols under the heading "Fraction" represent the fractions obtained by differential centrifugation and extraction of rat liver homogenates in a medium of 88M sucrose They stand, from top to bottom, for Nuclei, Nuclear Washings, Mitochondria, and Supernate The centrifuged nuclear washings consisted predominantly of mitochondria When the activity of these mitochondria was added to the activity of the bulk mitochondrial fraction "M,"

of the measurement, because one can easily vary the specifications of the microdiffusion units to meet a large variety of experimental requirements

For a number of reasons, tyramine was used as the standard substrate In trying to find a tyramine concentration that would be suitable for the purpose of assay, we encountered the difficulty illustrated in Figure 46. In this particular experiment, the enzyme source was a rat liver homogenate, but similar results were obtained with a number of organs from different animal species. At temperatures of incubation close to that of the body, other factors being equal, the average reaction velocity of this system is dependent upon the concentration of the substrate Under such conditions, one cannot very well measure how much enzyme there is in a given preparation However, if the temperature of the reaction is lowered, one gets the familiar Michaelis type of curve, with a comfortable plateau on which to work

The intimate details of this phenomenon are better illustrated in Figure 47 It is evident that below so-called physiologic tempera-

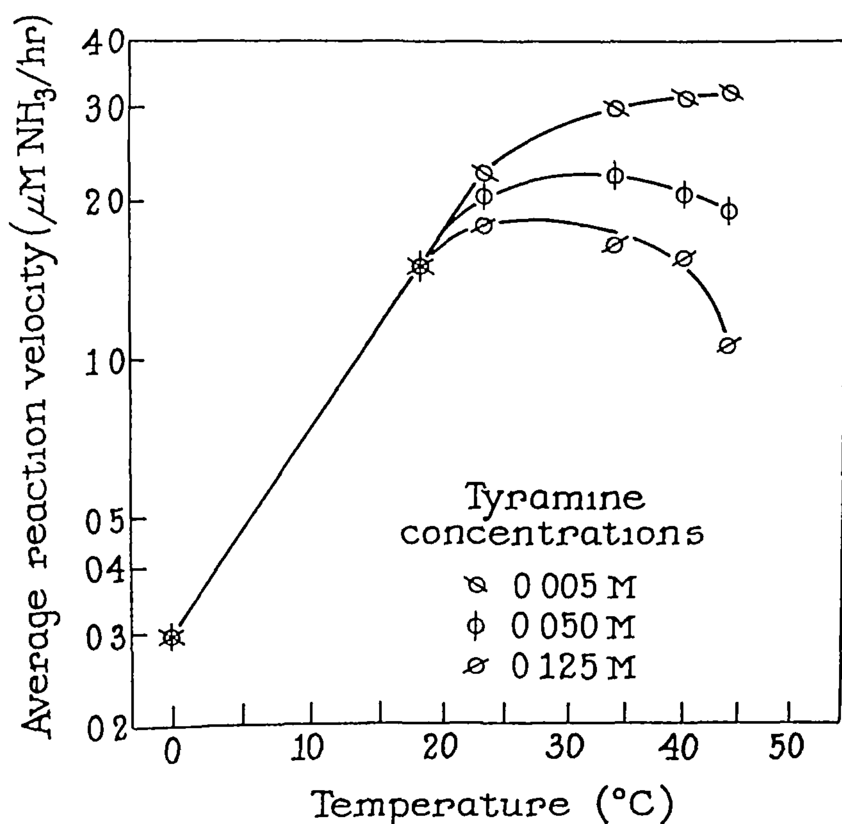


FIGURE 47 Reprinted, by permission, from Cotzias, G C, and Dole, V P Metabolism of amines *J Biol Chem* **190**, *665 (1951)*

tures, there is a wide range of substrate concentrations, the variation of which does not affect the performance of the enzyme Our working hypothesis on this phenomenon, which is not restricted only to tyramine, is that the amine promotes the thermal denaturation of this as well as other enzymes Be that as it may, this situation forced us to adopt lower temperatures of incubation than those conventionally used in this type of work, so as to avoid a multiplicity of forces which otherwise would adversely affect the average reaction velocity of this enzyme system. With these precautions, it is quite easy to determine accurately the activity of even small amounts of mono amine oxidase Furthermore, in the study of inhibitors, one can be more confident that the inhibition observed is due to the inhibitor, rather than to the substrate

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M	13 7±1 8	56 8	56± 15	26 4	26 ± 6 9	219± 25
S	1 9±1 2	7 9	.91± 33	42 9	2 6± 2	20± 14
Sum	23 8	99 5	2 07	97 6		
Homogen	24 1±2		2 12± 38		11 9± 2 6	

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17 amine oxidase units out of the 24 contained in one gram of rat liver were found associated with those bodies Furthermore, the satisfactory recovery of the homogenate's activity in the fractions demonstrates the validity of this measurement

By working with this procedure, a different sense of quantities is developed The question arises whether each of the enzyme "units" which are located in the cells of various organs, and in the various organelles of the same cell, performs the same functions I do not believe that our present data can fully answer this question They might, however, give us a measure of insight into the function of some strategically-located enzyme units, under specific conditions

In considering possible physiologic and pharmacologic functions of the *in vitro* substrates of this enzyme, one is struck by the fact that most of the substrates are potent sympathomimetic agents These amines produce measurable contraction of the terminal vascular bed If mono amine oxidase were present in the vascular bed, we thought we might be able to manipulate its potency and investigate the existence of a concordant variation in the sensitivity of the vessels to known *in vitro* substrates of this enzyme It appeared desirable to use enzymatic inhibitors which by themselves were as free as possible of sympathomimetic properties

TABLE XIV

Tissue	Amine Oxidase (Units per Gram Pooled Tissue)
Mesenteric Arteries	5 18
Mesenteric Veins	2 02
Mesenteric Membranes	1 24

Table XIV summarizes the results of enzyme assays on teased mesenteric arteries, veins, and relatively avascular membranes Comparison of the values obtained in this experiment with those found in studying the liver, indicates that 5 units of mono amine oxidase are present per gram of arteries, while with liver there were 24 units per gram, and with liver mitochondria two to three times that number Considering the total body mono amine oxidase, it is evident that the amounts contained in these vessels are indeed

small It is, however, obvious that mono amine oxidase is indeed present in blood vessels, being more concentrated in the arteries than the veins.

Nickerson Were the membranes fat-free?

Cotzias If you are thinking about the assay, it doesn't make any difference as far as we know

Nickerson. No, but it might make a big difference in units per gram

Cotzias Yes, indeed We cut out, very specifically, membranes that did not contain gross fat, whereas the arteries I am talking about here were teased from the fatty strands of the mesentery

In order to establish the next premise, we investigated the *in vitro* inhibitory effects of some substances which, if anything, have weak sympathomimetic characteristics We found that there are some such agents which definitely inhibit mono amine oxidase, and are in the process of looking for more Figure 48 shows the effect of indolacetic acid, in the form of its sodium salt, on the deamination of tyramine by a rat heart homogenate In this study, we had to

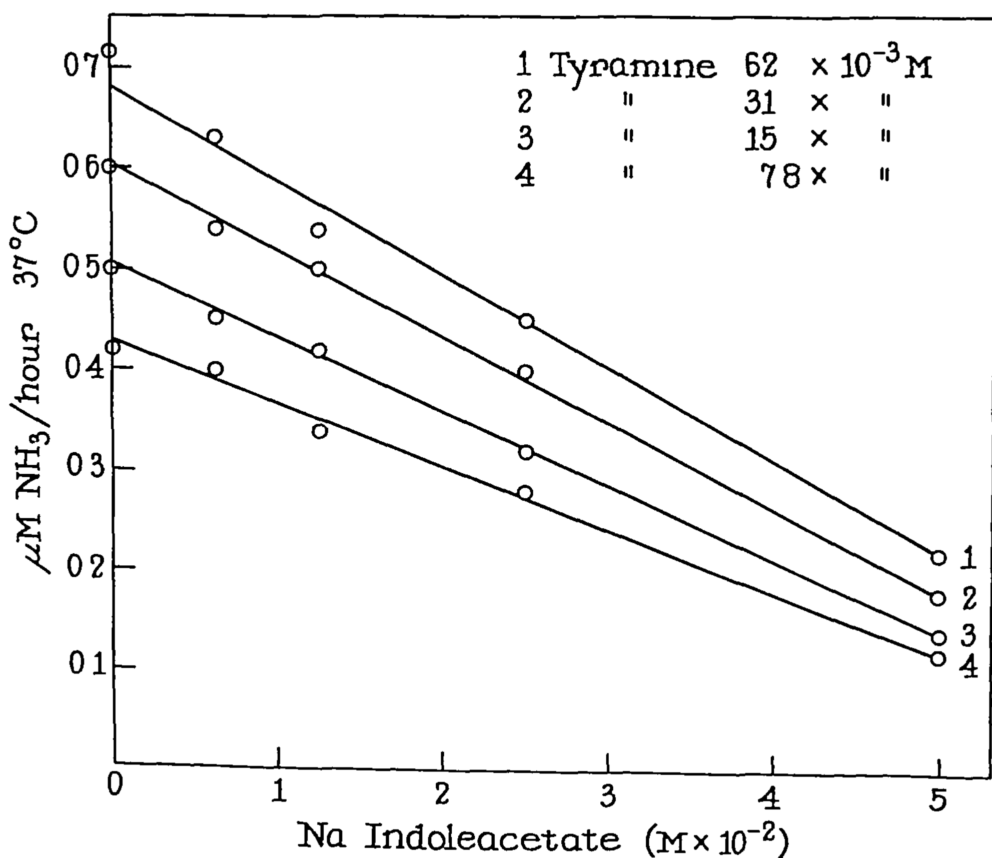


FIGURE 48

avoid the usual tenfold dilution in favor of twofold dilutions so as not to blot out the weak inhibitory capacity of this agent. The inhibitory effect is evident from the data It is of interest that this agent is a potent plant growth hormone, which, however, is encountered in mammalian urine, probably as a by-product of tryptophan metabolism

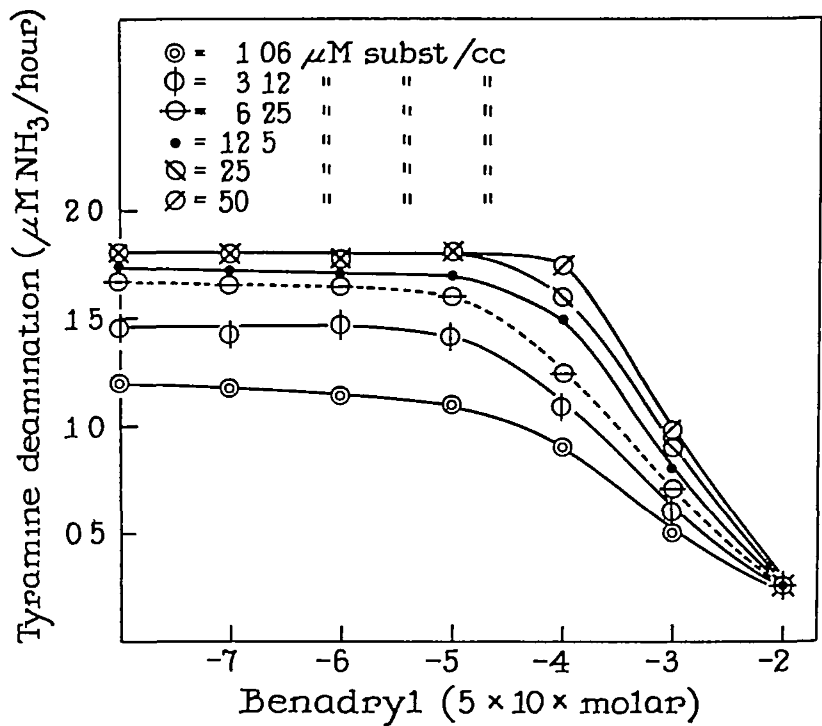


FIGURE 49

Figure 49 shows the behavior of Benadryl, which is chemically different from indolacetate compound This antihistaminic is a fairly potent, noncompetitive mono amine oxidase inhibitor In Figure 50 is summarized the evidence that the dye methylene blue is another *in vitro* inhibitor of this enzyme

The question now arises as to whether these agents are capable of inhibiting the enzyme in the intact animal following injection I am afraid that convincing proof of this cannot be shown in the case of indolacetate, probably because of the weakness of its inhibitory power With Benadryl, however, as can be seen in Figure 51, it is another story Plotted in this Figure are the results of experiments in which the antihistaminic was injected intravenously into comparable animals which were sacrificed by decapitation 15 minutes later The mesenteries of these animals were dissected,

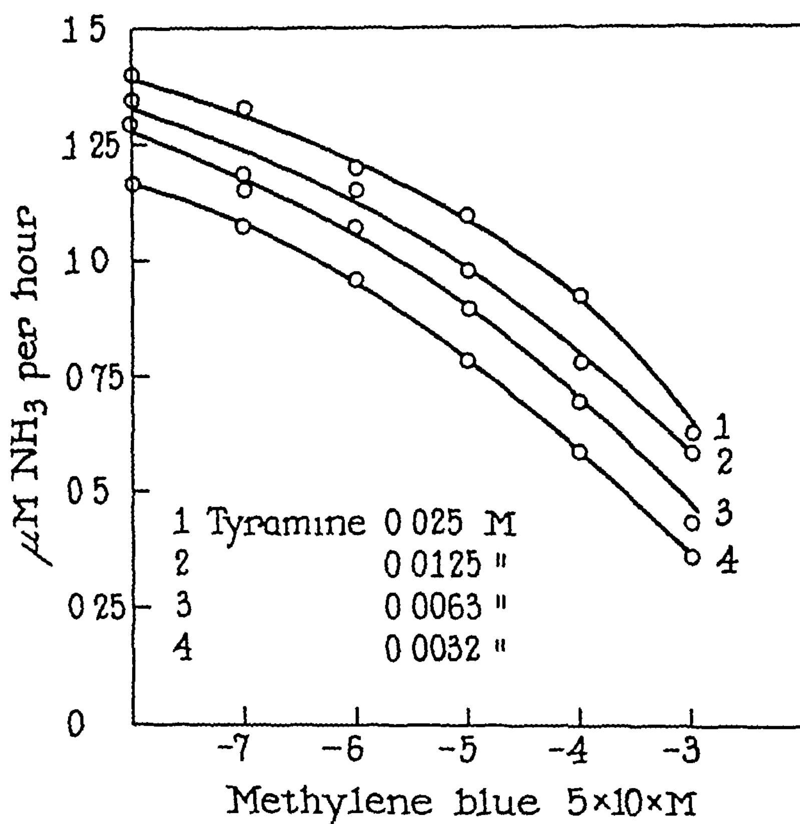


FIGURE 50

homogenized, and tested *in vitro* for enzymatic activity. There is a rough reverse proportionality between the amount of inhibitor injected into the animal and the rate of deamination of tyramine per unit of mesentery nitrogen. The same sort of phenomenon occurs with methylene blue, as shown in Figure 52.

To summarize, a procedure has been described for measuring the activity of mono amine oxidase in tissues, and some evidence given that the technique is a valid one It has been shown that mono amine oxidase exists in blood vessels and that it can be inhibited by the *in vivo* administration of inhibitors The question now arises whether this inhibition of the enzyme in the vessels is accompanied by some change in the way the vessels react to substances which are known *in vitro* substrates. To explore this, we relied on the well-known Chambers-Zweifach technique (Dr Theodore Balourdas of the Department of Biology at New York University performed many of the bioassays) This technique consists essentially in determining the concentration of, for example, epinephrine or nor-epinephrine, which will cause a definite constriction of the precapillary sphincters of the meso-appendix when

TABLE XV

***In Vitro* and *In Vivo* Comparison**

Inhibitor	Inhib Conc for 25% Inhibition *(In Vitro)*	Vascular Sensitization *In Vivo*		
		Amine	μM Inhibitor / 100 gm Rat	Sensitization
Indole -3- Acetic acid	5×10^{-2}	Adrenaline	64	20
		Nor-adrenaline	41	100
		Tyramine	61	10
Benadryl	5×10^{-4}	Adrenaline	10 2	25
		Nor-adrenaline	8 7	300
		Tyramine	9 3	10
Methylene Blue	5×10^{-5}	Adrenaline	2 4	25
		Nor-adrenaline	2 5	300
		Tyramine	2 8	10

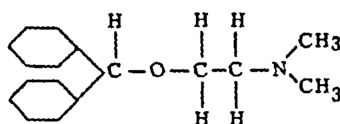
anesthesia, because our rats did not lie still with 4½ milligrams. We had to use about 6 milligrams.

The possibility that the *in vitro* and *in vivo* correlation could be fortuitous, and might not live up to a study which would employ a battery of inhibitors, led to the introduction of another kind of control This is shown in Figure 53 On top, the chemical formulae of the three inhibitors are shown These, as I said, were injected intravenously The chemical formulae of the amines applied locally for determination of the threshold are shown in the lower section These are divided into two categories in respect to their *in vitro* behavior towards mono amine oxidase substrates and nonsubstrates The threshold concentration of the substrates was lowered following the injection of the inhibitors

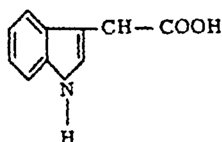
This change in threshold was evident, as previously noted, when the concentration of the locally applied amine had to be brought down to levels which would cause minimal precapillary sphincter constriction It was equally evident when the concentration of these amines was kept at the original threshold level Then, following the injection of the inhibitors, a marked prolongation of the time during which the vessels remained constricted was found The constriction was evident, not only in the precapillary sphincters, as was the case initially, but the entire preparation would blanch out, due to constriction of both arterioles and venules, which are normally quite

INHIBITORS INJECTED

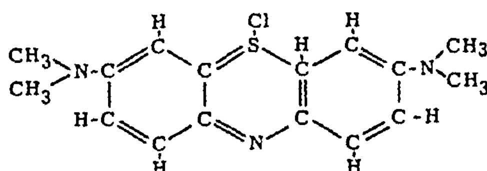
BENADRYL



INDOLE ACETIC ACID



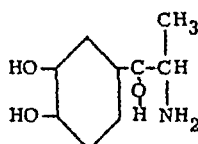
METHYLENE BLUE



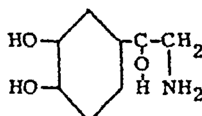
LOCALLY APPLIED

MAO SUBSTRATES

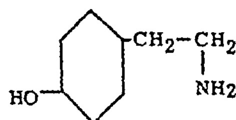
ADRENALIN



NORADRENALINE

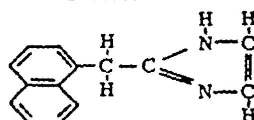


TYRAMINE

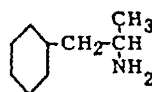


MAO NON SUBSTRATES

PRIVINE



BENZEDRINE



EPHEDRINE

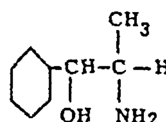


FIGURE 53

insensitive to these amines With most experiments, the increased sensitivity appeared a few minutes after the intravenous injection of the inhibitor, reached a maximum at about 15 minutes, and came back to normal about an hour later

In the case of the nonsubstrates, a different situation was encountered The initial threshold concentrations were higher, as a

rule, than those encountered with the previous group of amines With Privine, Benzedrine, and ephedrine, 1 per cent solutions had to be applied, and in some preparations even 10 per cent, in order to obtain constriction of the precapillary sphincters With epinephrine, a concentration on the order of 1.5 million (w/v) was usually needed, and with nor-epinephrine 1 1 million (w/v). The most interesting finding was, however, that after the injection of indolacetate, Benadryl or methylene blue, we were unable to detect any change in sensitivity to locally-applied Privine, Benzedrine, or ephedrine In other words, the vessels of the meso-appendix seemed to discriminate between amines which are substrates of mono amine oxidase, and those which are not, despite their chemical similarities of structure

Selkurt. May I point out the parallel in the experiments I showed, in that after denervation the response to nor-epinephrine seemed to be more enhanced Perhaps you could explain the increased sensitivity

Cotzias I have a fear of dragging innervation into this problem, because I am under the spell of Dr Lorente de Nó

Nickerson: I might add that Dr Burn has published data which he believes prove that changes in amine oxidase account for the increased sensitivity of denervated structures to nor-epinephrine They are not too convincing in my opinion

Cotzias. We interpret our data as strongly indicating that mono amine oxidase is a major *in vivo* pathway in the metabolism of such amines as epinephrine, nor-epinephrine, and tyramine. For the time being, I shall let it go at that

Shorr. Before we discuss this work, I would like to mention some of our very recent, and as yet incomplete, experiments which have an indirect bearing on the possible relation between mono amine oxidase inhibition, and the hyperreactivity of the meso-appendix vessels Our objective was to differentiate between hypertensin and VEM We had found previously that the injection of crude renin gave a delayed VEM response, but we could not tell whether VEM was present as an impurity in the renin, or whether renin, via hypertensin, was of itself exercising a VEM effect More recently, Dr. Harry Goldblatt very generously gave us a considerable supply of highly purified renin with an activity of 2700 dog units/mg. On injection, this highly purified preparation produced an immediate brief rise of blood pressure and, 18 to 30 minutes later, a delayed VEM effect. However, when the kidney was ligated before the

injection of renin, the VEM effect was abolished, although the blood pressure rose as before

A possible explanation is that renin brings about renal vasoconstriction, and some measure of renal hypoxia, a metabolic circumstance leading to the formation of VEM which is then released as the vasoconstriction wears off Assays of saline extracts of the renal cortex, excised 20 minutes or so after the intravenous injection of renin, showed that VEM was present in considerable concentrations in the kidney

It then occurred to us that the action of Benadryl, in increasing the sensitivity of the muscular capillary vessels to epinephrine, might likewise involve the renal VEM systems Exclusion of the kidney from the circulation 30 to 90 minutes prior to the injection of Benadryl likewise abolished, in a number of animals, the hyperreactivity observed to follow Benadryl when the kidney was in the circulation

EDITOR'S NOTE Dr Shorr wishes to add the following

An analysis of this phenomenon, subsequent to the Conference, appears to have disclosed some of the factors responsible for Benadryl-induced hyperreactivity in the muscular capillary vessels In amounts of 1 to 3 mg per 100 gm of body weight, Benadryl was found to lead to hyperreactivity only when the injection was followed by an asphyxial effect When the asphyxia was prevented by a previous tracheotomy, the threshold response remained unaltered In several animals, with previous ligation of the kidney, in which the injection of Benadryl was associated with an asphyxial response, no hyperreactivity was encountered Hence, part of the hyperreactivity with asphyxial doses of Benadryl would appear to be attributable to the concurrent renal hypoxia, with the formation and subsequent release of renal VEM Neurogenic discharge also contributes to hyperreactivity of the muscular capillary vessels following induced asphyxial reactions (unpublished studies) Hence, it would seem that the hyperreactivity of the terminal vascular bed, observed with these asphyxial doses of Benadryl, cannot be used in support of the concept that there has been an inhibition of mono amine oxidase at these peripheral sites

Nickerson I think one of the implications here is that all sensitizing procedures are dependent in some way upon the kidney I should like to dispel that point of view, at least with respect to Dibenamine, and Dibenzyline, and some of their degradation products We have obtained sensitization up to the point where they reacted to one to a billion epinephrine in animals in which we could not demonstrate VEM in the circulation, and also in arenal animals 12 hours after operation

Cotzias Dr Shorr, do you really mean to say that when there is no kidney, you do not get sensitization to injected Benadryl?

Shorr That is the case

Cotzias Have you tried using any of the methylene blue I have given Dr Baez?

Shorr. Not as yet.

Cotzias I am wondering what cocaine, or any one of the other agents in which one finds a concomitant inhibition of this system, would do in an arenal preparation Could you induce any other type of sensitization in the arenal animal?

Zweifach Surgical removal of the kidneys does not completely interrupt the development of vascular hyperreactivity Various agents, such as histamine, which produce vascular hyperreactivity upon intravenous administration, bring about the same vascular change in the nephrectomized, as in the normal animal Cortisone will increase the reactivity of the mesenteric vessels in nephrectomized animals When nephrectomized dogs are subjected to several types of acute stress, the terminal vascular bed in the omentum shows a significant increase in vascular reactivity For example, using anoxia, or excess CO_2, as the stress stimulus, a considerable potentiation of the vascular response to topical epinephrine is obtained in control animals The same experiment carried out in nephrectomized dogs produces a somewhat blunted hyperreactivity about 50 to 75 per cent of the reactivity values obtained in control experiments

Cotzias You mean to say that any sort of sensitization of the vascular bed is mediated through VEM?

Zweifach It is obvious that vascular reactivity is a result of the composite influence of a number of separate factors Surgical interference with each of these systems alters or blunts the response of the vascular bed to standardized stimuli As was indicated, the kidney is the source of a humoral principle, VEM, which potentiates the response of the terminal vascular bed to epinephrine When the kidney is removed, this vasoexcitor factor is obviously eliminated However, the response to other hormonal and neurogenic factors persists In addition, there are a great many local tissue factors which can increase the vascular reactivity, irrespective of the presence of the kidney All of these factors must be taken into consideration

Shorr All that we can say is that, under these specific circumstances, if we exclude VEM production by the kidney, the response of the meso-appendix vessels to injected renin is completely blunted

Cotzias It should be investigated whether VEM would account for the behavior of isolated organs? In those, also, there is a con-

siderable degree of sensitization by some of the agents that I have mentioned I should also be very much interested in finding out what the mesentery shows on testing *in vitro*, after the nephrectomy has been performed and these agents have been injected. We have demonstrated inhibition in an animal with intact kidneys, and I feel that before it is taken for granted that the phenomenon is not as highly specific as I still think it is, it should be demonstrated that it exists in the preparations you have just described

Shorr I should like to ask Dr von Euler to summarize and complete his discussion, to the extent that he can, in the remaining time

NOR-EPINEPHRINE IN SHOCK

von Euler Since this Conference is on shock, I thought it appropriate to collect some data on the effect of nor-epinephrine, and epinephrine, in conditions of shock I should like to add that in this work I have not had any personal experience My information is what I have collected from colleagues and from the literature.

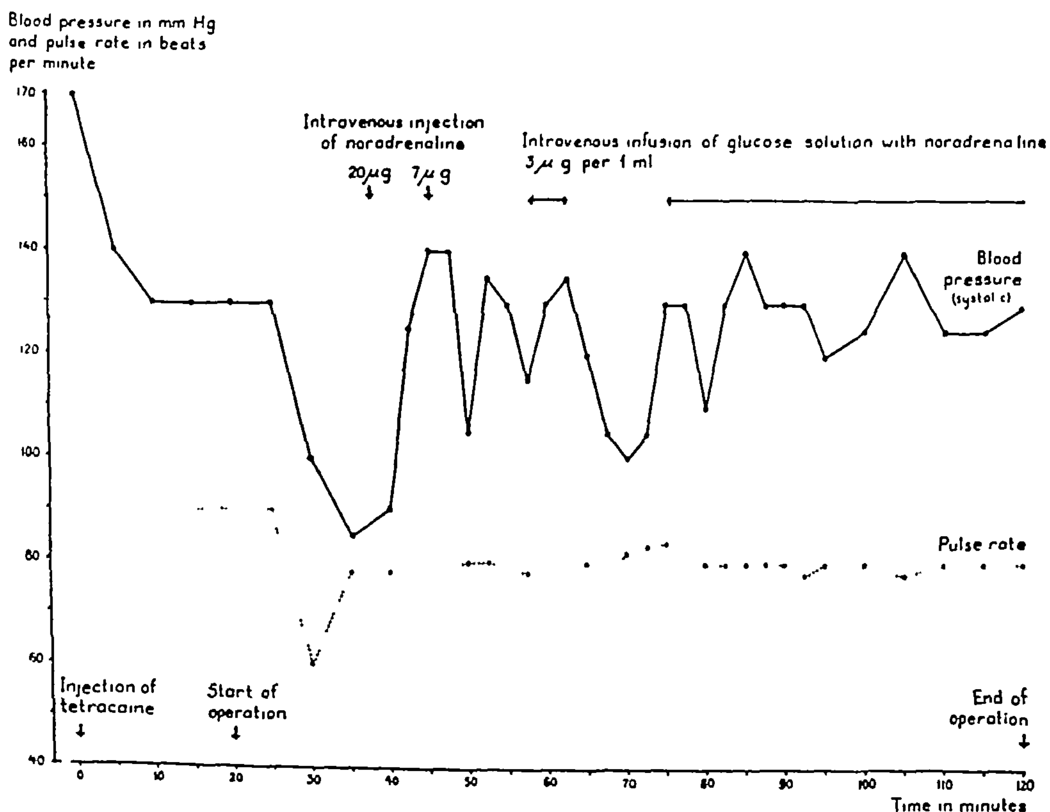


FIGURE 54 Effect of nor-epinephrine in a case with a serious fall in blood pressure during spinal anesthesia (68-year-old male) Reprinted, by permission from Arner, O Complications following spinal anesthesia *Acta chir scandinav* **Suppl. 167** *(1952)*

Figure 54, taken from a paper by Arner (73), shows the effect of nor-epinephrine in spinal anesthesia It seems that when an operation is started, there is a drop in blood pressure down to about 80, and then after a few single injections, and after saline infusion of nor-epinephrine, the pressure goes up and is kept up. As Dr. Goldenberg and his co-workers have shown (74) blood, in many cases, is not sufficient to raise the pressure in states of shock; whereas, when nor-epinephrine is given in addition, it is capable of maintaining the blood pressure

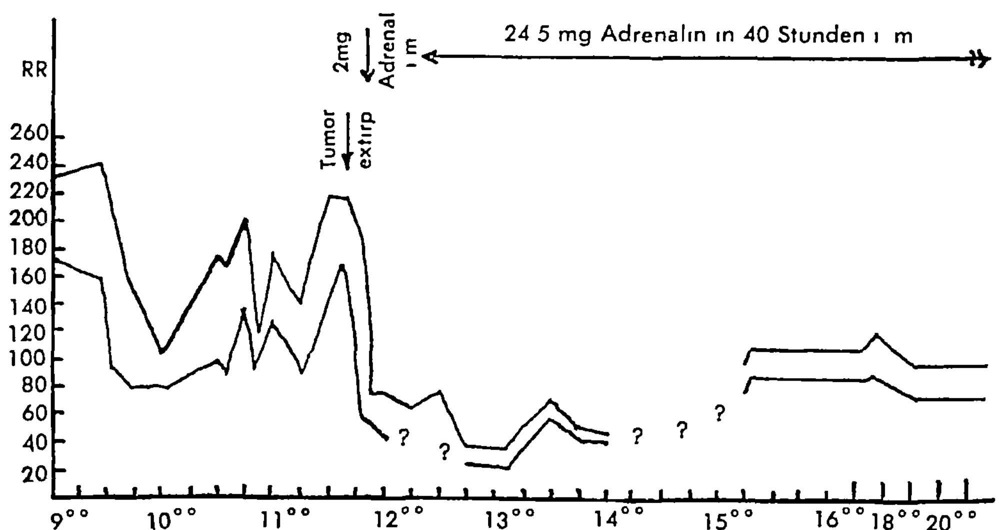


FIGURE 55 Blood pressure of patient from which a phaeochromocytoma was removed Sharp drop in pressure on removal of tumor Unsatisfactory action of 2 mg epinephrine i m every 5 min for 1¼ hour Reprinted, by permission, from Peiper, H J *Ueber Phaochromocytome* Diss Med Fak. Univ , Mainz, 1952

Figure 55 is a case of pheochromocytoma, observed by Peiper (75) After the removal of the tumor, the pressure went down, as it very often does, to low levels In this case, epinephrine was given, and in no less a quantity than 24 5 mg in 40 hours The effect was conspicuously quite small on the blood pressure, and one would almost be tempted to say that the patient survived, or came out of the shock, in spite of the epinephrine I have the impression from what I have heard from colleagues that epinephrine may be quite dangerous in conditions of shock similar to this

Nelson Dr von Euler, isn't this because for the patient, blood flow is the most critical consideration? Since blood flow is proportional to pressure, and inversely proportional to the resistance, when the resistance is increased and the pressure decreased, the flow might actually be cut down

von Euler That is very possible, I should say. But there seems to be a difference in the action of epinephrine and nor-epinephrine in this respect In several of the cases of pheochromocytoma, which I was referring to this morning, nor-epinephrine was given, and with a very striking result when the pressure was low. In one of these cases, it had to be given for 24 hours at the rate of 1 mg per hour That kept the blood pressure at about 120 mm Hg, and after that the patient came around with a steady blood pressure. In one case in which nor-epinephrine was given, the patient was operated at a time when the old method, if I may say so, of epinephrine was used In that case, the pressure never rose again

Nickerson One thing which we might get into the record is an item which came up at this same hour at the session last year That is the data on protection by vasoconstrictors If we limit ourselves to data for which there are adequate controls, I cannot think of a single experiment in which, in shock produced by any means, the administration of a vasoconstrictor to raise the blood pressure has increased the survival rate On the contrary, the great majority of these experiments indicate that vasoconstrictors lower survival rate

EDITOR'S NOTE Dr von Euler wishes to add here an "after thought" to the effect that perhaps nor-epinephrine is a particularly suitable vasoconstrictor

Remington Dr Nickerson, you are speaking of dog shock, and perhaps not of the consequences of human syncope

Nickerson Dog shock, or rat shock, or any kind of shock in which we have a sufficiently standard degree of trauma so that we can know, at least statistically, whether the subject would have survived without treatment Other observations are not conclusive We never know how badly off the patient is, and we never know whether the patient would have survived had a particular drug not been given I believe that last year Dr Stead proposed that we classify these as Class A, Class B, Class C, and Class D clinical observations a very useful classification

Nelson There may be an indication for the use of a vasopressor drug as protection against the post hypercapnic phenomenon (76) characterized by a fall in blood pressure and cardiac irregularities, after there has been a change from a high alveolar carbon dioxide pressure to a normal or to a low pressure The individual will have a fall in blood pressure which may be a precursor of actual cardiac arrest In dogs, at least, if a vasopressor is given, some protection is afforded

Figure 54, taken from a paper by Arner (73), shows the effect of nor-epinephrine in spinal anesthesia. It seems that when an operation is started, there is a drop in blood pressure down to about 80, and then after a few single injections, and after saline infusion of nor-epinephrine, the pressure goes up and is kept up As Dr Goldenberg and his co-workers have shown (74) blood, in many cases, is not sufficient to raise the pressure in states of shock; whereas, when nor-epinephrine is given in addition, it is capable of maintaining the blood pressure

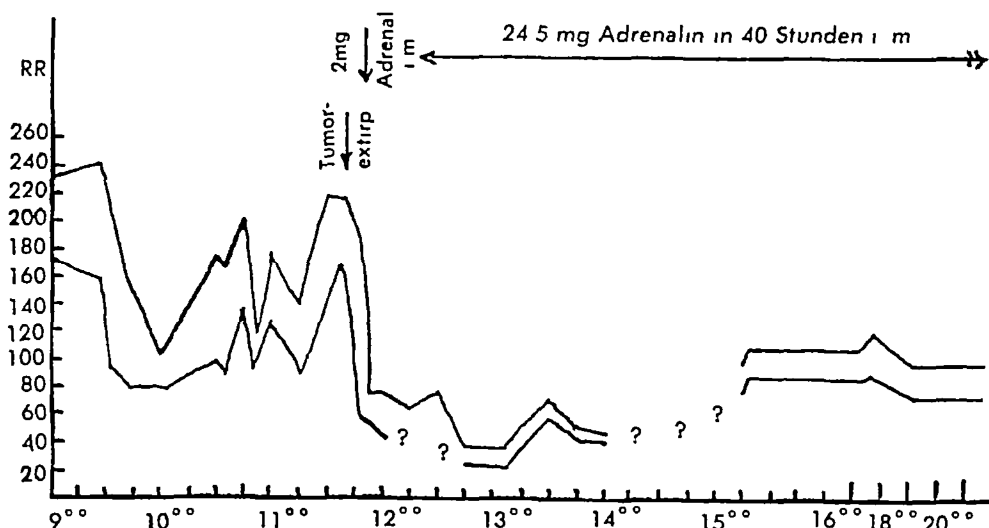


FIGURE 55 Blood pressure of patient from which a phaeochromocytoma was removed Sharp drop in pressure on removal of tumor Unsatisfactory action of 2 mg epinephrine i m every 5 min for 1¼ hour Reprinted, by permission, from Peiper, H J *Ueber Phaochromocytome* Diss Med Fak Univ, Mainz, 1952

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Nelson Dr von Euler, isn't this because for the patient, blood flow is the most critical consideration? Since blood flow is proportional to pressure, and inversely proportional to the resistance, when the resistance is increased and the pressure decreased, the flow might actually be cut down

There is a fairly convincing paper along these lines coming from the Halloran and Brooklyn Veterans Administration Hospitals (77). They used nor-epinephrine in diverse types of shock and it worked very well. I admit that this is a purely empirical paper. Blood-flow studies in vital organs, such as the liver or kidney, were not done, and these naturally have to be done to prove the point that in spite of the evidence of all the dog experiments, raising the perfusion pressure with nor-epinephrine might help to increase the blood flow in vital organs of patients in shock.

Dr. Bradley (78) thought that at a low blood pressure level, e.g., under conditions of high spinal anesthesia, there might be an increase of renal blood flow with nor-epinephrine, instead of the decrease observed with nor-epinephrine at normal pressure levels, because the influence of the increased perfusion pressure outweighs the local vasoconstriction. I am fully aware that all of the Dibenamine experiments on pre-treatment and prevention of shock give just the opposite story, but I wonder what these Dibenamine experiments mean. For example, Dr. Remington's paper (79) describes the best protection as being provided by 5 mg. Dibenamine, administered shortly before the dog was started bleeding. Is that correct?

Remington: Yes.

Goldenberg: The epinephrine response at this dose was still preserved, not fully reversed, as happens with higher Dibenamine doses. I wonder whether the response to nor-epinephrine and to sympathetic nerve stimulation, was tested under the same conditions. With this Dibenamine dose, the humoral effect of epinephrine and nor-epinephrine may be blocked, and the neurogenic vasoconstriction may not be able to be blocked. I think that this point was completely disregarded in the papers on Dibenamine. But I would not try to shift the whole story to the dog, as Dr. Ahlquist did. The essential point is that nor-epinephrine, and epinephrine, actions differ so much from species to species that investigations cannot be limited to one species and the results applied to another, e.g., man. If I may make a humble suggestion, the dog seems to me to be the most unsuitable species for this type of investigation.

EDITOR'S NOTE. Because of lack of time, the following material was not presented at the Conference, but is added here at Dr. von Euler's request:

Considerable evidence has already accumulated to show that nor-epinephrine may be useful in conditions of shock. Among those conditions the following may be enumerated: postoperative shock (74,80,81), barbiturate poisoning (82), extirpation of pheochromocy-

Nickerson I think one might focus attention on the cardiac stimulant effect in those cases.

Richards: There are two other indications that I can think of. One is severe barbiturate poisoning, and the other is drowning or immersion. When the respiration has ceased and blood pressure falls away to nothing, the prognosis is quite bad If the patient is then sustained by neosynephrine, continuous infusion, and artificial respiration for 48 hours, interrupted from time to time, and still the blood pressure falls, and respiration has not quite recovered, if after that time the reflexes return and the patient does begin to breathe again, which has happened in more than one instance – I know of two barbiturate poisonings and one drowning that we have had – then I feel that the sustaining of the blood pressure over that period of time has had a life-saving effect

Nickerson. This discussion fulfills one of the purposes of the Conference It indicates experiments which need to be done If, in groups of 20 animals carried to a comparable point of depression, the survival is actually better with a vasoconstrictor plus oxygen than with oxygen alone, the point you make will be established

Remington· Or with a cardiac stimulant.

Nickerson· What do you mean?

Remington With the use of a drug which is not a vasoconstrictor, but a cardiac stimulant

Nickerson There is much to be said for that, and I think we have the pharmacologic tools to evaluate the cardiac factor One might compare, in parallel groups of animals, the effects of isopropylnor-epinephrine, epinephrine, and nor-epinephrine

Richards Epinephrine does not work very well, or did not with us, in those circumstances, because it increased the pulse rate so much.

Goldenberg I wonder whether the dog is a suitable animal with which to decide the problem of the use of nor-epinephrine in shock Dr Ahlquist made the point that epinephrine is a much stronger vasoconstrictor than is nor-epinephrine, i e , in the dog's exteriorized spleen I would not say that this is proof that it is the transmitter in the dog, but it shows the striking difference in the hemodynamic response to nor-epinephrine between dog and man In my opinion, it stresses the fact that the crucial experiments must be done in man, e g , having a patient in hemorrhagic shock who does not respond to fluid replacement and, after repeated attempts at increasing the volume of blood infused, a switch is made to nor-epinephrine and the patient recovers.

blood pooled somewhere in the organism It seems most probable that this is in the venous system, a view held by many authorities on shock

There is good evidence that this may actually be the case. With the aid of radioactive isotopes, Nylin and Pannier (89) studied the mixing time in various conditions, and found that in shock it may be greatly prolonged (Figures 56 and 57). Ordinarily, a complete mixing,

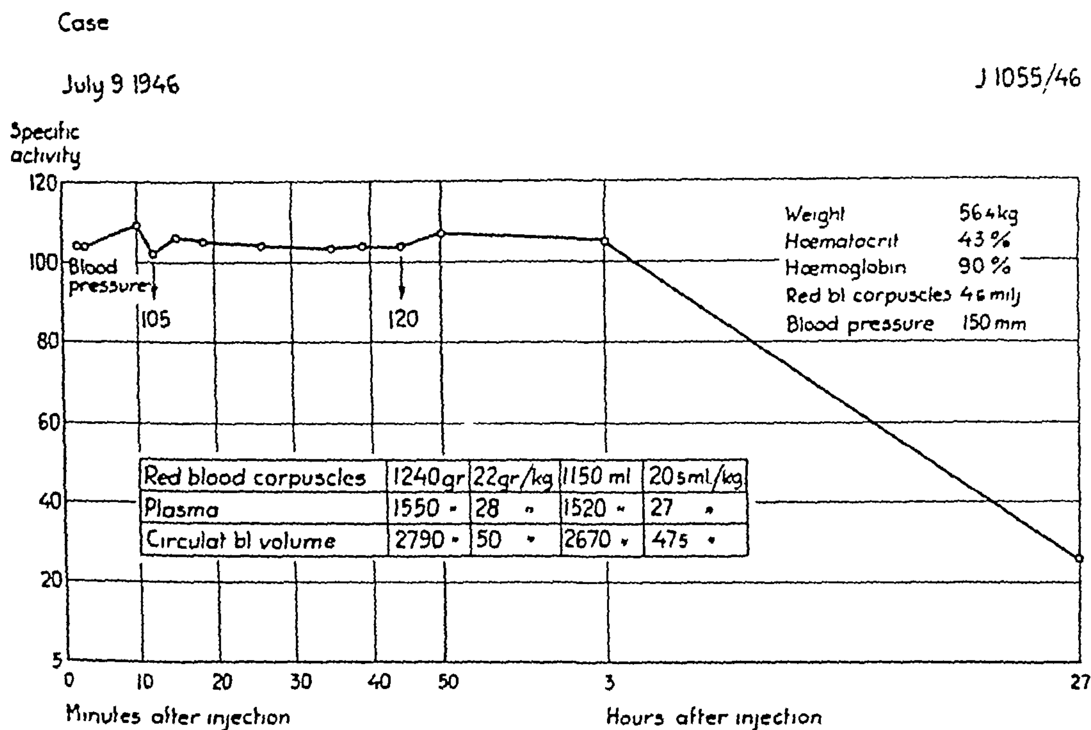


FIGURE 56 Specific activity of blood in patients with spinal anaesthesia without (A) and with (B) shock Note that equilibrium is reached very slowly in (B) Reprinted, by permission, from Nylin, G, and Pannier, R L'influence de l-orthostatisme et du shock sur la vitesse circulatoire determinée à l'aide du phosphore radioactif *Arch internat de pharmacodyn et de thérap* **73**, *401 (1947)* Reprinted, by permission, from Nylin, G, and Pannier, R Influence of orthostatism and shock on circulation speed, determined by aid of radioactive phosphorous *Nord med.* **34**, *1065 (1947)*

as judged from the attainment of an equilibrium in the specific activity of the blood, occurs within a few minutes, while in shock mixing may not be complete until after 40 minutes Obviously, the total red corpuscle figures, calculated from the specific activity at 5 or 10 minutes, would correspond to much less than the real volume of blood. The implication is that blood is pooled in the vascular system in such a way that it does not take part in the circulation at the normal rate It would seem plausible that this blood is lingering in the venous system, probably largely in the visceral areas The slowly decreasing figures for the specific activity in the experiments referred to indicate that the blood is not completely shut off, but simply moving very slowly

toma (83,84), coal gas poisoning (85), spinal anesthesia (73), hypotonia after hexamethonium, myocardial infarct (86,82), postural hypotension, and after adrenalectomy (87)

Goldenberg and his group (74) were the first to use intravenous infusions of nor-epinephrine as a therapeutic measure in shock, they reported favorable results in 1949 Since then, the effectiveness of nor-epinephrine in combating shock has been repeatedly confirmed

Mayer and Ruben (80) described an illustrative case in whom shock came 36 hours after a severe abdominal operation The patient received glucose containing epinephrine, and whole blood in adequate quantities, but without effect After several hours of unsuccessful treatment, the blood pressure was still 60/40 mm Hg, and the heart rate 160 per minute About 12 hours after the onset of shock, the patient was given an intravenous infusion of a solution containing 4 mg of *l*-nor-epinephrine per 1000 milliliters The blood pressure rose within one minute to 102/64 and the patient became conscious During the next four hours, his blood pressure was maintained with nor-epinephrine, a total of 10 mg , at 104-124/70-86 Neosynephrine was not effective It is often necessary to continue an intravenous nor-epinephrine drip for a prolonged period in order to secure a good final result

The beneficial effect of nor-epinephrine in restoring the blood pressure in certain conditions of shock may be due to a favorable effect on the heart, on the vessels, or on both The reason why epinephrine is not capable of restoring, or maintaining, the blood pressure as effectively as nor-epinephrine, might be explained by the vasodilator effect of epinephrine in important vascular areas, together with its strong central and synaptic depressant action In addition, the effects of epinephrine on the heart (such as tachycardia, and wasteful oxygen consumption) may be regarded as undesirable

Theoretically, nor-epinephrine should be more suitable for raising low blood pressure than epinephrine, since it is the natural vasoconstrictor mediator and raises the mean pressure without interfering with the metabolism to any marked degree There is reason to assume that the moderate store in the suprarenals represents a reserve for emergency blood pressure homeostasis

There are many points which merit consideration in this connection There is hardly any need to emphasize that no vasoconstrictor drug can be expected to do much good if a severe loss of fluid has occurred, or if severe anoxia prevails Only if the blood pressure persists at shock levels after the fluid losses have been compensated and oxygenation is adequate, might one expect favorable results from nor-epinephrine

It has been repeatedly claimed on theoretical grounds that there is no rationale for the treatment of low blood pressure in shock with vasoconstrictor drugs, since there is evidence of arterial constriction in shock This opinion may be based on an erroneous interpretation of the actual observations If the cardiac output is low, the filling of the arterial tree will be inadequate and a moderate vasoconstriction will keep the vessels narrow, or even shut, if the blood pressure is below the critical closing pressure (88) If, on the other hand, gross fluid losses have not occurred, there must be a considerable quantity of

5 SWANSON, E E , and CHEN, K K Pressor activity of *l*- and *d*-nor-epinephrine, U S P reference standard epinephrine, and synthetic *l*-epinephrine *J Am Pharm A (Scient Ed)* 41, *187 (1952)*
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20 WADA, M , SEO, M , and ABE, K Effect of muscular exercise upon the epinephrine secretion from the suprarenal gland *Tohoku J Exper Med* 27, *65 (1935)*

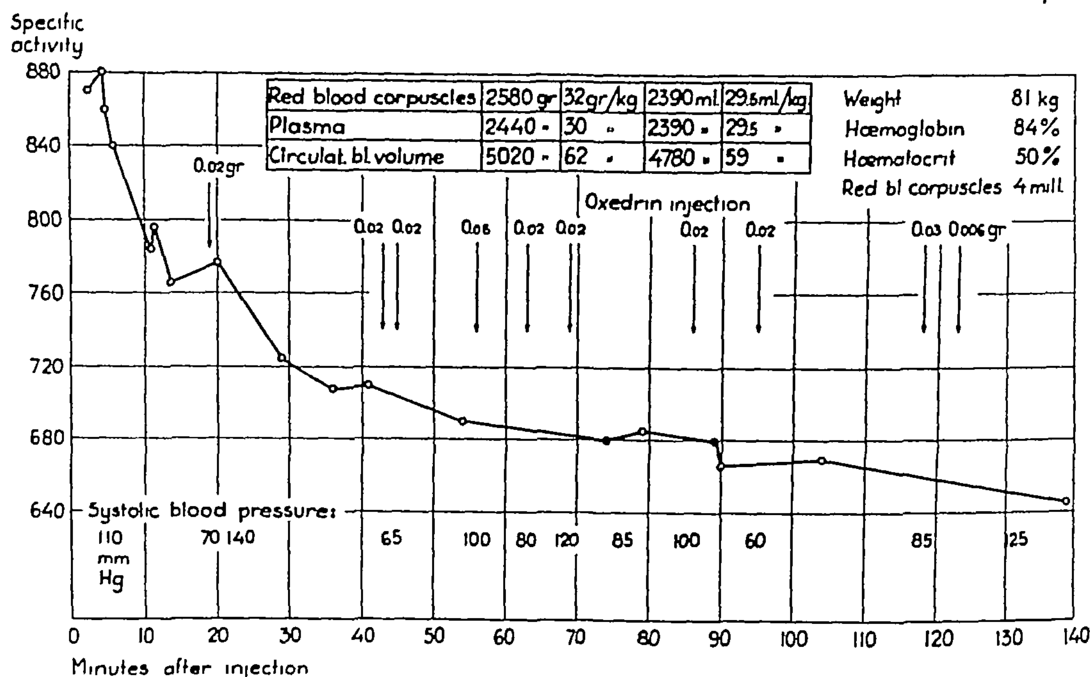


FIGURE 57

Unfortunately, we know very little about the cause of this tendency of the blood to pool outside the effective circulation Clearly, many factors would have to be considered toxic influences, local anoxia in the vascular areas, etc Assuming this condition of stagnation in the veins to be characteristic of shock, how would the effect of nor-epinephrine be explained? It seems to me that nor-epinephrine might, in sufficient doses, constrict the venules and larger veins to such an extent that the venous return would be increased and the heart, if still in a good condition, could increase its stroke volume and fill the arterial tree The concomitant arterial constriction would then help to maintain a good blood pressure level So long as nor-epinephrine, or any similarly acting drug, was administered, improvement could be maintained until the underlying cause of the venous pooling had passed

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